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# THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

## THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXXXVI—No. 2

*Issued September 1, 1928*

BALTIMORE, U. S. A.

1928

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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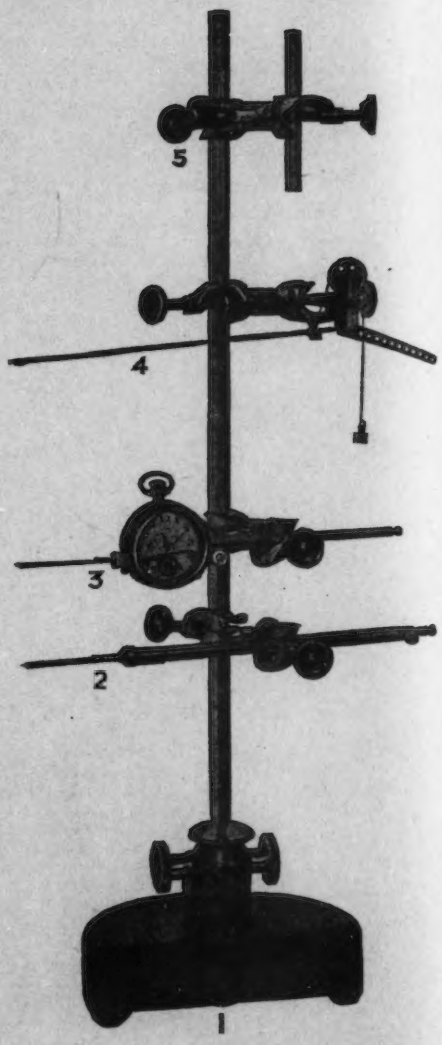


## JOSEPH BECKER

**630 West 168th Street** **New York City**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 86 SEPTEMBER 1, 1928 No. 2

# THE DISTRIBUTION OF THE ESTRUS-PRODUCING AND ESTRUS-INHIBITING HORMONES IN THE OVARY OF THE COW

W. B. PAYNE, H. VAN PEENAN AND G. F. CARTLAND

*From the Research Laboratories, The Upjohn Company, Kalamazoo*

Received for publication June 20, 1928

Many well-confirmed experiments have proved the presence of an estrus-producing hormone in the ovary, follicular fluid and placenta. Reports regarding the presence of this hormone in the corpus luteum have shown less agreement. Parkes and Bellerby (1) have recently offered an explanation for this disagreement. They found that the small amount of estrus producing hormone usually found in mixed samples of corpus luteum is quantitatively located in the enclosed fluid of hollow specimens. These authors conclude that the estrus producing hormone is not elaborated by the corpus luteum but is merely mechanically enclosed in a small percentage of the glands.

Evidence is constantly accumulating that the corpus luteum elaborates a hormone which inhibits ovulation and estrus. Pearl and Surface (2) inhibited ovulation in the fowl by injecting emulsions of desiccated corpus luteum. Papanicolaou (3) reports delayed estrus in the guinea pig after injection of corpus luteum extracts, but does not give his method of extraction. Working with mice, Loewe and Lange (4) found only slight indications of delayed estrus after injection of fat soluble extracts of corpus luteum in amounts representing 36 grams of fresh corpus luteum per mouse. Parkes and Bellerby (1) have recently succeeded in inhibiting estrus in the non-castrated mouse by injecting the acetone soluble fat of cow's corpus luteum. These authors report that the inhibiting hormone in the corpus luteum is labile and that active extracts rapidly deteriorate even when preserved in the cold.

The estrus producing hormone when obtained by extraction with fat solvents has been found to be in the unsaponifiable fraction. It would seem possible that the inhibiting hormone might also be localized in the unsaponifiable fraction of the fat of corpus luteum. With this point in

view we have studied the distribution of the estrus producing and estrus inhibiting hormones between the unsaponifiable fractions of the acetone soluble fats of ovarian residue and corpus luteum.

EXPERIMENTAL. The samples of corpus luteum and ovarian residue used in the following experiments represented carefully collected, hand-dissected material, dried immediately after collection in a high vacuum and below 40°C. Only ovaries containing corpora lutea were used in these experiments, the blanks being rejected. The corpora lutea represent a mixture of corpora lutea of ovulation and of pregnancy. The samples were taken from a large number of cattle and are representative of material found on the pharmaceutical market. The material for extraction was ground to pass a no. 20 sieve.

*A. Isolation of the unsaponifiable fractions.* Five thousand six hundred seventy-three grams of desiccated corpus luteum yielded by extraction 720 grams of phosphatide-free fat. The methods of isolation and a complete chemical analysis of this fat are reported in an earlier paper (5). Saponification of 700 grams of this fat, representing 5515 grams of desiccated corpus luteum, yielded 93 grams of unsaponifiable material containing 62 grams of cholesterol. Fifty-eight grams of cholesterol were separated by crystallization from alcohol and the mother liquor concentrated to a volume of 53 cc. and preserved in nitrogen-filled ampoules. Each gram of solids in this solution represents 158 grams of dried corpus luteum. This material was dissolved in olive oil for injection.

Six thousand one hundred thirty grams of desiccated ovarian residue yielded by extraction 261 grams of phosphatide-free fat. A complete chemical analysis of this fat has been reported (6). Saponification of 247 grams of this fat, representing 5801 grams of dried ovarian residue, yielded 46.3 grams of unsaponifiable material from which 30.8 grams of cholesterol were separated by crystallization. The concentrated alcoholic mother liquor was ampouled in an atmosphere of nitrogen. Each gram of solids in this solution represents 374 grams of dried ovarian residue. This material was dissolved in olive oil for injection.

*B. Unsaponifiable fractions tested on spayed rats.* These fractions prepared in like manner from dried corpus luteum and dried ovarian residue were assayed for the estrus producing hormone using the method of Allen and Doisy (7). When injections were made subcutaneously in olive oil, the active dose was the same when given in a single injection as when distributed in three injections at four hour intervals. The results are given in table 1.

The unsaponifiable fraction of corpus luteum fat is active in 180 mgm. doses. A similar fraction from ovarian residue is active in 40 mgm. doses. Calculated in terms of dried gland, the corpus luteum contains 35 rat units per kilogram and ovarian residue 66 rat units per kilogram. Since ex-

tractions were made from the dried gland, these values are not interpreted as maximum yields of estrus-producing hormone. Higher yields are usually obtained from the fresh tissue. These results do, however, indicate the distribution of estrin between the corpus luteum and ovarian residue in the composite sample of ovaries examined.

TABLE 1

*Extracts tested on spayed rats*

| SOURCE OF MATERIAL | DOSE | NUMBER OF RATS INJECTED | NUMBER POSITIVE | NUMBER NEGATIVE |
|---|---|---|---|---|
| | *mgm.* | | | |
| Corpus luteum | 300 | 1 | 1 | 0 |
| Corpus luteum | 180 | 6 | 6 | 0 |
| Corpus luteum | 135 | 10 | 2 | 8 |
| Corpus luteum | 90 | 2 | 0 | 2 |
| Ovarian residue | 240 | 2 | 2 | 0 |
| Ovarian residue | 160 | 4 | 4 | 0 |
| Ovarian residue | 120 | 1 | 1 | 0 |
| Ovarian residue | 80 | 6 | 5 | 1 |
| Ovarian residue | 40 | 6 | 4 | 2 |
| Ovarian residue | 20 | 2 | 0 | 2 |

TABLE 2

*Assay of extracts by premature puberty test*

| SOURCE OF MATERIAL | TOTAL DOSE | NUMBER OF RATS INJECTED | AGE WHEN INJECTED | AGE AT PUBERTY | AGE OF CONTROLS AT PUBERTY |
|---|---|---|---|---|---|
| | *mgm.* | | *days* | *days* | *days* |
| Corpus luteum | 180 | 4 | 25 | 32 | 95–100 |
| Corpus luteum | 135 | 4 | 31 | 36 | 95–100 |
| Corpus luteum | 90 | 2 | 31 | 90 | 85–87 |
| Corpus luteum | 60 | 2 | 33 | 60 | 68 |
| Ovarian residue | 160 | 1 | 30 | 34 | 91 |
| Ovarian residue | 120 | 2 | 30 | 34 | 91 |
| Ovarian residue | 80 | 1 | 30 | 34 | 91 |
| Ovarian residue | 40 | 2 | 30 | 34 | 91 |

*C. Unsaponifiable fractions tested by the premature puberty method.* Allen and Doisy (8) produced puberty in rats as early as 26 days of age by the injection of 3 to 20 rat units of follicular hormone. We were interested in comparing the minimum active dose of estrus producing hormone by this method and the spayed rat method. Female rats of 25 to 38 days of age were injected on three successive days with the unsaponifiable fractions of corpus luteum and ovarian residue. The minimum dose which induced

premature puberty was determined using non-injected litter mates as controls. Opening of the vagina and a positive estrus smear were taken as criteria of sexual maturity. Results are given in table 2. One rat unit of estrus producing hormone injected into rats 25 to 33 days old induces puberty in 4 to 7 days. Non-injected controls became sexually mature at 68 to 100 days of age.

*D. Tests for the estrus-inhibiting hormone.* The unsaponifiable fractions from corpus luteum and ovarian residue were injected into normal, sexually mature, virgin guinea pigs and the effects on the estrus cycle observed. Two cycles were observed as controls and on the 9th and 10th days of the third cycle, subcutaneous injections of the extracts were made. The pigs

TABLE 3

*Tests for the estrus—inhibiting hormone*

| SOURCE OF MATERIAL | TOTAL DOSE | LENGTH OF NORMAL ESTROUS CYCLES | LENGTH OF ESTROUS CYCLE AFTER INJECTION | LENGTH OF SUBSEQUENT ESTROUS CYCLES | ACTIVITY OF EXTRACT (MGM. PER UNIT*) |
|---|---|---|---|---|---|
| | *mgm.* | *days* | *days* | *days* | |
| Corpus luteum | 600 | 16, 16 | 20 | 16 | 150 |
| Corpus luteum | 900 | 15, 15 | 21 | 16 | 150 |
| Corpus luteum | 1,200 | 15, 15 | 25 | † | 120 |
| Ovarian residue | 600 | 14, 15 | 16 | 15 | |
| Ovarian residue | 1,000 | 15, 16 | † | | |
| Ovarian residue | 1,300 | 15, 15 | † | | |
| Cod liver oil | 400 | 15, 15 | 16‡ | 16 | |
| Cod liver oil | 800 | 15, 15 | 17‡ | 15 | |

* Papanicolaou's unit = one-fifth of the dose which delays estrus in the guinea pig for 5 days.

† Guinea pig died without coming into estrus.

‡ Slightly toxic; guinea pigs lost 30 to 65 grams in weight during 7 days following injections.

were examined daily by the vaginal smear method and the delay in the occurrence of estrus determined. A subsequent estrus cycle was also measured as a control. Papanicolaou (3) has defined the inhibitory unit as one-fifth of the dose which delays estrus in the guinea pig for 5 days. The results are summarized in table 3.

The unsaponifiable fraction of corpus luteum is non-toxic in 600 to 900 mgm. doses and has an activity of one inhibitory unit per 150 mgm. Higher doses show corresponding activities but are toxic causing severe abscesses and rapid loss of weight. The unsaponifiable fraction of ovarian residue is non-toxic in 600 mgm. doses and is lacking in the estrus inhibiting hormone. No significance can be given to the one day delay in estrus in

the guinea pig receiving the 600 mgm. dose. Higher doses of ovarian residue unsaponifiable are toxic causing rapid loss in weight and obliteration of estrus. In this case the failure to come into estrus is due not to a specific inhibitory hormone but to general toxic effects. The cholesterol free unsaponifiable fraction of cod liver oil was injected as a control. The slight delay in estrus observed in these two pigs is attributed to the transient toxic effects following injections of this material.

There is still some question as to whether the estrus-inhibiting hormone acts directly upon the vagina or whether it acts indirectly through the ovary by inhibiting ovulation. Loeb and Kountz (9) report that both hormones of the ovary inhibit ovulation when injected into normal ovulating guinea pigs, but that they produce opposite effects upon the estrus changes in the vagina. The estrus inhibiting action observed in our experiments is obviously not due to the same hormone which produces estrus in the spayed rat. Our extract of ovarian residue has over four times the estrus producing activity as a similar extract of corpus luteum, but contains no demonstrable estrus-inhibiting activity. The fact that we observe a diestrus period of normal duration following the delayed estrus would indicate that the inhibitory hormone acts upon the ovary. Our results indicate that the estrus inhibiting hormone of the ovary is localized in the corpus luteum.

## SUMMARY

The cholesterol-free unsaponifiable fractions of the fat of corpus luteum and ovarian residue have been assayed for their estrus producing and estrus inhibiting hormones. In terms of dried gland the corpus luteum contains 35 rat units and ovarian residue 66 rat units of estrus producing hormone per kilogram. The minimal active dose of unsaponifiable material was 180 mgm. for corpus luteum and 40 mgm. for ovarian residue. The corpus luteum contains 42 guinea pig units of inhibitory hormone in the unsaponifiable fat from one kilo of dried gland. The inhibiting hormone is lacking in a similar extract of ovarian residue.

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# STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

## XXIII. Growth of the Gonads and Bursa Fabricii in Doves and Pigeons, with Data for Body Growth and Age at Maturity

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Received for publication June 14, 1928

Once the bursa Fabricii has come under sufficiently close study in birds of known age it turns out that its growth, and its involution, closely coincide with the following events: It grows while the thymus grows; while the body is *rapidly* growing; and while growth in the *gonads* is obviously repressed. It begins its involution when the thymus begins involution; when adult weight is nearly attained; and when the gonads begin a more rapid development. The involution of the bursa is usually completed—that is, it usually disappears—coincident with sexual maturity. It tends to persist longest in birds which mature latest. These associations seem to suggest an endocrine function for this particular organ.

Growth phenomena in the gonads of the two sexes present two very interesting conditions. First, they show a remarkable dissimilarity of size at the end of embryonic life. Though the adult testis is three to five times heavier than the adult (resting) ovary, the ovary of the female embryo at hatching is 63 to 100 per cent larger than the testis of the male embryo of the same age. These facts raise the presumption that something associated with the *embryonic* life of the bird either favors ovarian development or restricts testicular development. This presumption is much supported by the fact that the adult bird gonad—particularly the testis—readily exhibits very marked size reduction under a wide variety of conditions. A specific reason for this size difference between ovary and testis at the end of embryonic life is sought in the fact that the follicular hormone is now known to be present in the egg-yolks, and this really implies that all embryos are subjected to an unavoidable and continuous dosage with this hormone. If the hormone affects the embryonic gonads in the same way it appears to affect testis and ovary in early post-natal stages, the observed size relations of the embryonic testis and ovary are those which we should expect. The second point of interest in our data on gonad growth in the bird can be more briefly stated. A post-embryonic period of repressed

gonad growth occurs coincidentally with the development of the bursa—as stated in an earlier paragraph—and this repression applies both to ovarian and to testicular development.

Growth curves for all stages of the development and involution of the bursa Fabricii are presented here for the first time. These are supplied for birds belonging to two different zoölogical families (*Columbidae, Peristeridae*)—the common pigeon (*Columba*) and the ring dove (*Streptopelia*). Complete growth curves for ovary and testis are also first supplied for these two quite different species. Polygons giving the distribution of age at maturity in the females of common pigeons, ring doves, and in two kinds of dove hybrids, are included in this study. Also, data sufficient to show the exceptional rate of body growth in doves and pigeons were obtained from the birds used in this study.

MATERIAL AND METHODS. The common pigeons used here are essentially similar to the laboratory animals now available in most parts of the world. None of the highly specialized races—such as Runts, Fantails, Magpies, Pouters, Tipplers, etc.—are included, neither in our studies on growth nor on age at maturity. The ring doves, and the ring dove hybrids, of this study require a more extended statement. The ring doves are the equivalent of the small domesticated, brown ring dove (*Streptopelia risoria*) now kept in captivity in all parts of the world. Many of those used by us did indeed contain the blood of the nearly related white ring dove (*St. alba*); but this contamination is frequent and widespread among the brown doves of the world, and is unimportant to the present studies. The "ring dove hybrids" of this paper are formed by those ring doves described above which had traces (one-sixteenth or less) of oriental turtle dove (*Turtur orientalis*) blood in them. The data from "ring doves" and "the ring dove hybrids" were separately tabulated. It was then found that in regard to growth of body, bursa and gonads the two are equivalent and can be combined. In our study of "age at maturity," where a much larger number of females is involved, a slight difference in the two groups is indeed evident (fig. 8). The study on "age at maturity" includes also a fourth group of doves called "trispecifics." These birds were essentially "ring doves," but they carried some blood of the Japanese ring dove (*St. douraca*). The birds used had not been much or long inbred. Nearly all of the data presented here were obtained prior to June, 1925.

The birds were killed at all months of the year. The size of the gonads of adult birds undergoes some seasonal change (Riddle, 1925), and a seasonal effect is probable also in the juvenile and newly hatched stages though this point has not been definitely determined. Fewer birds aged less than 5 months were killed during April, May and June than at other seasons. All the birds used were protected from severe cold; and they fed upon the same grains at all seasons. The weights of birds aged less than 5 months

are net weights after deducting the contents of the crop. Embryo weights are those obtained after removal of unabsorbed yolk from the body cavity. The gonads and bursa were taken from birds freshly killed by decapitation. The weights of the smaller glands were obtained on a balance sensitive to one-tenth milligram. The ovaries of adult birds, before weighing, were freed from those occasional ova (4+ mm. diameter, and with yellow yolk) which had entered upon the final period of rapid growth. Such ova would normally be present in the pigeon ovary not more than one-fifth of the days of adult life; and these must, when present, be removed if truer and more comparable figures are to be obtained.

All birds with obvious disease were excluded from these data, since the bursa particularly was observed quickly to undergo temporary involution in disease or fasting. From a few of the older birds the data for bursa weight were not obtained; from some of the very young birds first examined by us no data for gonad size were taken. It thus results that for certain age-groups unequal numbers of these two organs were weighed. The body weights tabulated are the averages for the total number examined in each age-group.

It should be noted that age is not calculated from the time of hatching, but from the beginning of embryonic development—which coincides with the date on which the egg bearing this embryo was laid. In the case of the pigeon this method of reckoning age is possible, and it presents obvious advantages over the method used with mammals. In some respects it is the only proper method to use in comparative studies on birds, since time of hatching here makes no distinction between the widely divergent altricial and precocious forms. Moreover, it is expected that studies on the growth of body and organs will ultimately be made for the later days of the embryonic period. It is obviously better to have a single method of reckoning the progress of a process that is certainly continuous. The ring dove is already 14 to 15 days (0.5 month) old at hatching; the common pigeon is 18 days (0.6 month) old. If age, as calculated here, is to be converted into age as usually calculated for the fowl, it is thus clear that this involves a subtraction of 0.5 or 0.6 month from the ages assigned by us.

PRESENTATION OF DATA. Combined data for ring doves and ring dove hybrids are given in table 1, and the data for common pigeons in table 2. Growth in the bursa, gonads and body will here be discussed separately.

*Growth and involution of the bursa.* The size of the bursa, at all ages, is practically the same in the male as in the female (figs. 1–3). Its largest size is reached in ring doves of both sexes at 3.1 months; in common pigeons this is attained a little earlier—at 2.8 or 2.9 months. Bursa involution begins soon after the highest bursa weight is attained—at least in many or most of those birds which are to become sexually mature 2 or 3 months later. The curves are to be interpreted with this fact in mind. The

tabular data show that the bursa had entirely disappeared in several birds aged less than 6.0 months. It is, however, the *average* size of the bursa that is expressed in the tables and curves. In this average the total number

TABLE 1

*Growth of body, bursa Fabricii and gonads in ring doves and in ring dove hybrids (two groups combined)*

| SEX | AGE | BODY WEIGHT | BURSA FABRICII | | GONADS | |
|---|---|---|---|---|---|---|
| | | | Number | Weight | Number | Weight |
| | *months* | *grams* | | *mgm.* | | *mgm.* |
| Males | 0.5 | 5.6 | 34 | 3.3 | 18 | 0.60 |
| | 0.8 | 59 | 5 | 62 | 3 | 2.3 |
| | 1.15 | 98 | 14 | 121 | 6 | 3.1 |
| | 1.3 | 120 | 6 | 131 | 2 | 4.2 |
| | 1.7 | 134 | 7 | 200 | 7 | 5.6 |
| | 2.26 | 152 | 11 | 239 | 10 | 6.56 |
| | 3.1 | 156 | 10 | 263 | 8 | 10.7 |
| | 3.74 | 151 | 10 | 171 | 9 | 40.9 |
| | 4.7 | 151 | 7 | 132 | 8 | 227 |
| | 6.1 | 153 | 19(16)* | 68 | 20 | 531 |
| | 8.06 | 155 | 55(33) | 18.4 | 62 | 995 |
| | 11.75 | 160 | 104(14) | 2.6 | 110 | 1,160 |
| | 18.0 | 165 | 32(3) | 0.5 | 38 | 1,214 |
| | 23.4 | 170 | 18(1) | 5.0 | 20 | 1,261 |
| | 44.1 | 166 | 29(0) | 0.0 | 42 | 1,332 |
| Females | 0.5 | 5.3 | 34 | 3.3 | 16 | 1.27 |
| | 0.85 | 69.4 | 5 | 83 | 2 | 5.2 |
| | 1.1 | 100 | 6 | 113 | 4 | 7.2 |
| | 1.76 | 129 | 7 | 172 | 3 | 12.3 |
| | 2.55 | 140 | 7 | 208 | 7 | 17.5 |
| | 3.1 | 146 | 11 | 250 | 9 | 24.0 |
| | 3.64 | 143 | 11 | 213 | 6 | 40.3 |
| | 4.54 | 145 | 13 | 167 | 10 | 63.5 |
| | 5.6 | 149 | 13(9)* | 66 | 12 | 99.7 |
| | 6.2 | 153 | 19(16) | 51 | 17 | 187 |
| | 7.94 | 154 | 39(23) | 26 | 56 | 260 |
| | 12.0 | 160 | 72(9) | 3 | 87 | 315 |
| | 18.0 | 169 | 17(0) | 0 | 15 | 349 |
| | 23.2 | 157 | 12(0) | 0 | 13 | 461 |
| | 46.1 | 165 | 20(1) | 1 | 14 | 466 |

* The number of cases in which bursa tissue was actually present is given in parentheses.

of birds in an age-group are included, irrespective of the complete absence or the reduced size of the bursa in some of them. The relatively few birds aged 5 to 6 months that had no bursa were sexually mature. It thus be-

comes evident that in an individual bird which matures late (e.g., 10 to 12 months) the bursa does not diminish in weight according to the form indicated on the curve, but rather tends to retain a larger proportion of its former weight.

TABLE 2

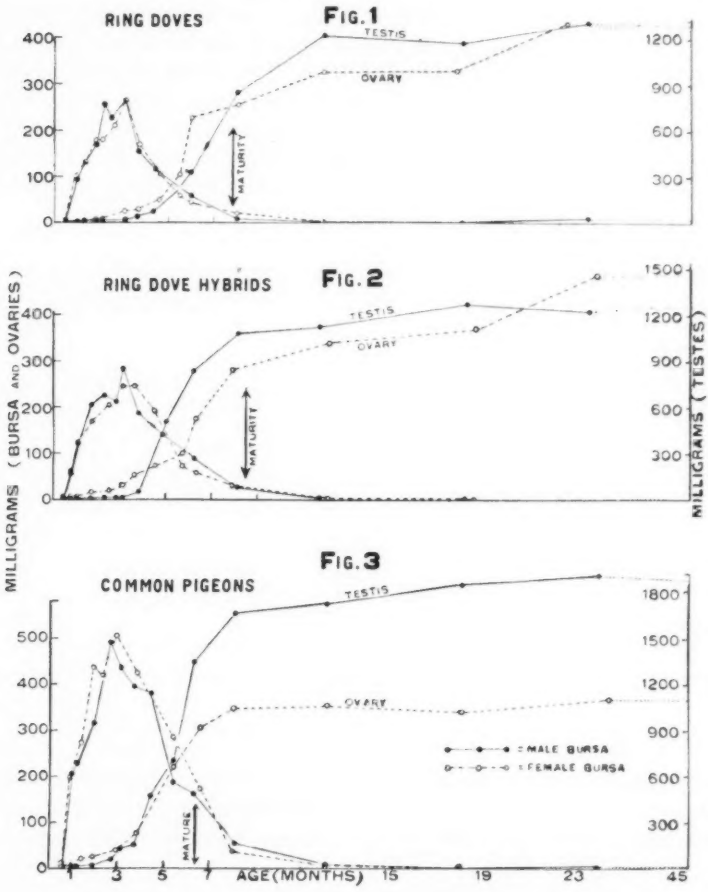
*Growth of body, bursa Fabricii and gonads in common pigeons*

| SEX | AGE | BODY WEIGHT | BURSA | | GONADS | |
|---|---|---|---|---|---|---|
| | | | Number | Weight | Number | Weight |
| | *months* | *grams* | | *mgm.* | | *mgm.* |
| Males | 0.6 | 9.9 | 13 | 12 | 13 | 1.5 |
| | 1.0 | 168 | 8 | 215 | 4 | 6 |
| | 1.2 | 228 | 8 | 229 | 3 | 7 |
| | 1.95 | 301 | 5 | 316 | 3 | 13 |
| | 2.7 | 332 | 3 | 490 | 4 | 56 |
| | 3.1 | 339 | 7 | 435 | 10 | 121 |
| | 3.7 | 327 | 4 | 394 | 9 | 151 |
| | 4.4 | 348 | 3 | 380 | 7 | 475 |
| | 5.4 | 381 | 4 | 188 | 18 | 704 |
| | 6.3 | 332 | 10(6)* | 162 | 11 | 1,347 |
| | 8.1 | 342 | 10(3) | 55 | 17 | 1,671 |
| | 12.1 | 347 | 54(5) | 9 | 62 | 1,721 |
| | 18.0 | 344 | 12(0) | 0 | 12 | 1,848 |
| | 24.0 | 378 | 11(1) | 2 | 10 | 1,913 |
| | 42.2 | 367 | 8(1) | 2 | 8 | 1,772 |
| Females | 0.6 | 9.9 | 12 | 10.2 | 10 | 2.4 |
| | 0.9 | 158 | 5 | 203 | 2 | 12 |
| | 1.4 | 273 | 8 | 274 | 3 | 19 |
| | 1.9 | 292 | 4 | 401 | 4 | 24 |
| | 2.3 | 288 | 6 | 436 | 2 | 35 |
| | 2.9 | 300 | 5 | 501 | 4 | 42 |
| | 3.8 | 337 | 5 | 424 | 3 | 75 |
| | 5.4 | 321 | 5 | 287 | 5 | 221 |
| | 6.6 | 309 | 13(7)* | 174 | 8 | 306 |
| | 8.0 | 331 | 9(2) | 37 | 14 | 349 |
| | 12.1 | 325 | 35(3) | 5 | 42 | 354 |
| | 18.0 | 343 | 8(1) | 3 | 8 | 340 |
| | 24.5 | 364 | 9(0) | 0 | 6 | 367 |
| | 43.0 | 336 | 10(0) | 0 | 10 | 364 |

* The number of cases in which bursa tissue was actually present is given in parentheses.

The bursa and the thymus show an interesting parallelism both in their growth and in their involution. This is well shown in figure 4. But there is also a notable difference. Normally the bursa completely disappears in all old birds; the thymus is merely reduced in size in old birds. The

figure also makes it clear that the beginning of involution in both bursa and thymus in ring doves is coincident with the end of rapid increase in body weight. Precisely the same is true for common pigeons; this is




Figs. 1–3. Growth curves

shown by a comparison of the present data with curves for the growth and involution of the pigeon thymus earlier published by Riddle and Frey (1925).

The growth of the bursa in terms of percentage body weight is shown in figure 5. It will be observed that the time of its maximum absolute size

is also the time of its highest percentage of body weight. The vertical arrows labelled "maturity" in figures 1 to 5 are placed at the point on the age-scale at which the greatest number of females become sexually mature—as shown by the production of their first eggs (see fig. 8).

The variability of the weight of this organ prevents a present decision as to whether the "late-maturing" birds show a lag in the attainment of their highest bursa weight; and also whether it is this full weight or a slightly reduced one that tends to persist for a very appreciable period. The curves truly show the course of bursa involution in a population, not in an individual. Since, however, the attainment of greatest bursa weight considerably precedes the time at which any bird becomes sexually mature,

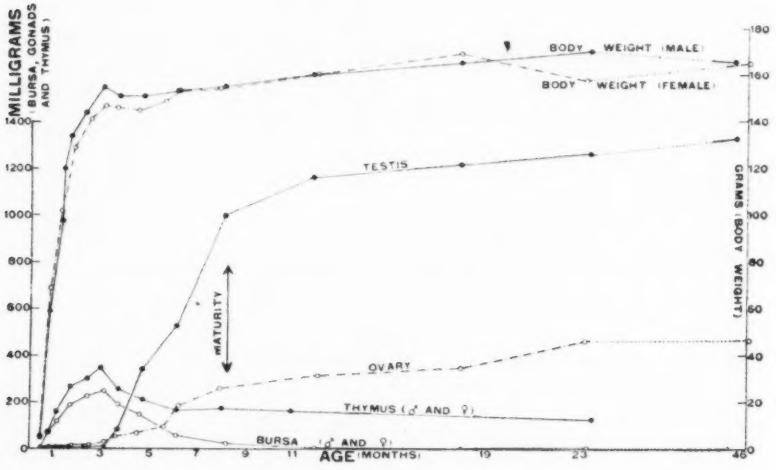



Fig. 4. Growth of body, gonads, bursa, thymus (ring doves)

it is evident that the beginning of involution is a well-marked period which distinctly precedes the attainment of sexual maturity. Indeed, when the bursa begins its decline a distinct and new step is taken toward the attainment of sexual maturity.

In some birds aged more than 6 months the size of the bursa presents a much more complicated situation than that which we have just described for younger stages and for typical adult stages. This complication must now receive some consideration. The bursa occasionally persists in birds of advanced age, including some cases in which the bird had long since become mature—as this was shown by the production of eggs. We do not understand these last-named cases. It has also proved difficult to find a short and convenient method of presenting these aberrant conditions.

A correlation table (fig. 6) provides one means of getting a look at these cases. The data of this table include *a*, females only; *b*, only those female ring doves and hybrids which *had* a bursa and were 6 to 11 months old; *c*, only cases obtained during 3 selected years (including 1927). The immature females are represented by circles; the 26 mature females by solid dots. The weight of the bursa is here plotted against the size of the ovary. In the case of the immature females the distribution suggests a negative correlation between weight of ovary and weight of bursa; though one extremely aberrant case is present. In general, this mildly reflects the situation found among doves and pigeons of all species at the age of 3 to 6 months. For the mature birds of the correlation table, however, it is clear

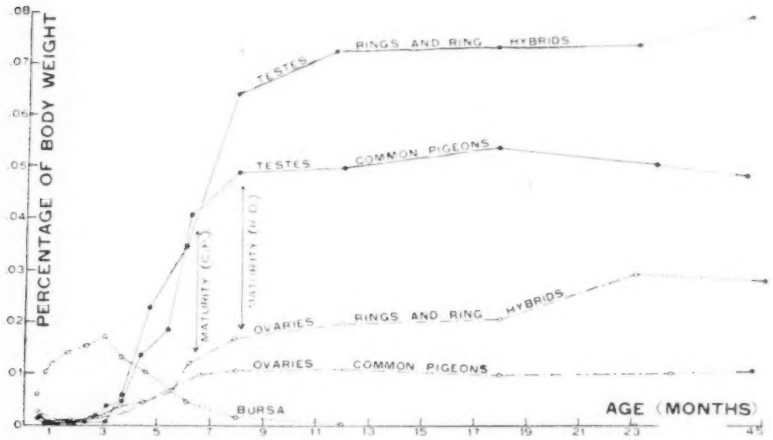



Fig. 5. Growth as percentage of body weight

only that large bursa size may coexist with large size of ovary. At present we can merely suspect that these rather rare cases of persistent bursa are quite analogous to status thymicus in the human.

It is clear that the involution of the bursa proceeds nearly or quite equally in the two sexes. Fairly adequate numbers are available for the groups aged 12 months. Among 54 male common pigeons there were 5, or 9.3 per cent, which still retained some bursa tissue; of the 35 females there were 3, or 8.6 per cent. Again, 14 of 104 (13.5 per cent) male ring doves, and 9 of 72 (12.5 per cent) females, had incompletely involuted bursa. These and other comparisons that may be made within the data of tables 1 and 2 show that this involution is quite independent of sex.

*The growth of testis and ovary.* Both testis and ovary grow very slowly dnring the rapid growth of the bursa (thymus and body). This is shown

for three kinds of doves and pigeons in figures 1 to 3. *The beginning of involution in the bursa (and thymus) marks the period of a much accelerated rate of growth in both testis and ovary.* Here also the acceleration shown by the tables and curves is for a population, not for an individual. As in the case of stages of bursa involution, noted above, the true slope of the curve for gonad growth in the early-maturing birds is unquestionably steeper than is indicated by our curves; for these curves are based upon

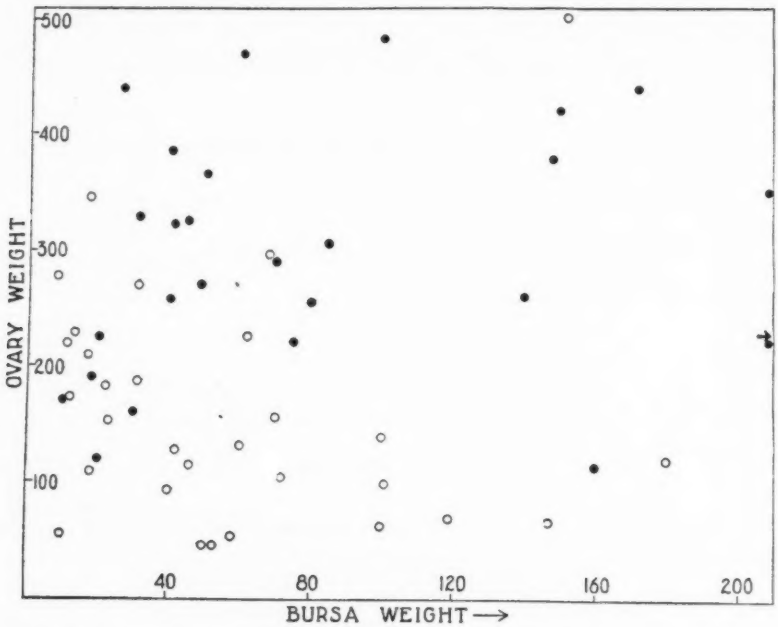



Fig. 6. Distribution of ovarian size and bursa size in cases of persistent bursa in ring doves aged 6 to 11 months. Solid dots represent mature females; circles, immature females.

groups—all of which probably include some early-maturing and some late-maturing individuals, and in these latter the gonads certainly grow less rapidly than they do in early-maturing birds.

Calculated on the basis of "percentage of body weight" (fig. 5) it is again made clear that *there is repression of gonad growth during the period of bursa growth. During bursa involution there is acceleration of gonad growth.* These same curves bring out the fact that in all mature stages (after 6 months) the gonads of common pigeons are smaller, as measured by per-

centage body weight, than are the gonads of ring doves. The common pigeons are birds of much larger body size, but it is interesting to observe that in the older females (over 18 months) the ovary is absolutely smaller than that of the ring doves (tables 1 and 2). The gonads show the smallest percentage of body weight at the time the bursa (also thymus) has its highest percentage of the body weight. When curves are made for absolute gonad weight during the first four months—and enlarged as in figure 7—the acceleration of growth beginning at or near the age of 3.0 months is clearly shown. In all of the four cases the greatest increase—the steepest segment of the point-to-point curves—begins with the point that is nearest to 3.1 months (ring doves) or to 2.8 months (common pigeons).

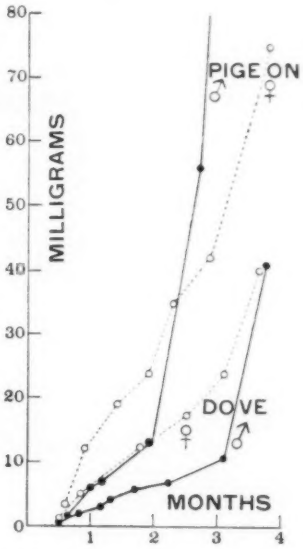



Fig. 7. Showing absolute gonad growth during the period of repression; and acceleration of growth (steepest slope) beginning at point measured nearest to age of 3.0 months.

From the period of hatching onward to the age of 3 months it is evident that *something associated with the general development imposes a restriction upon the growth of the gonads. At 3 months this restricting influence is eliminated or reduced.* Only at 3.0 months (2.8 months in common pigeons) do either testes or ovaries again attain to as large a percentage of the body weight as they had at hatching (0.5 or 0.6 month). After the lapse of another 3 months, i.e., at the age of 6 months, this percentage is increased 8 to 50 times.

It is of particular interest and importance that *at hatching the testes are only about one-half as large as the ovaries.* Such a difference was found in the three kinds of pigeons studied. In ring doves we get 0.6 mgm. for testes, 1.3 mgm. for ovaries; in ring dove hybrids, 0.6 for testes, 1.2 for ovaries (these two are combined in table 1, but separate in table 3). In the common pigeons we obtain 1.5 mgm. for testis, and 2.4 mgm. for ovaries. This is the reverse of the size relationships of these organs in the adult—where the testes are 3 to 5 times the larger. Moreover, the male embryos which supplied these data averaged slightly larger than the female embryos in all of the three groups studied. These facts are best shown in table 3.

The true interpretation of the data for growth of the gonads, and par-

ticularly for the growth and involution of the bursa Fabricii, can only be obtained in material in which the age at maturity is approximately known. We therefore submit data on this point in the form of distribution polygons in figure 8. It will be observed that the common pigeons tend to mature somewhat earlier (mode at 6.0 to 6.5 months) than the other kinds of doves. In these same birds we have noted that the bursa begins its involution, and the gonads begin an increased rate of growth, somewhat earlier than in ring doves. The ring dove hybrids are probably the least homogeneous of the four groups examined, and the data suggest two modes or centers of distribution in the polygons (average, about 7.5 to 8.0 months). The ring doves show a mode at 7.5 to 8.0 months; the trispecifics, at a similar age.

*Growth of body.* Point-to-point curves for the growth of male and female ring doves which supplied the data for the above-described studies

TABLE 3

*Size differences in testes and ovaries in the adult and at hatching*

| KIND OF BIRD | SEX | WEIGHT OF: | | |
|---|---|---|---|---|
| | | Adult gonad | Body weight of hatching | Gonad at hatching |
| | | *mgm.* | *grams* | *mgm.* |
| Ring doves | Male | 1,300 | (5.60) | 0.61 |
| | Female | 432 | (5.13) | 1.30 |
| Ring hybrids | Male | 1,420 | (5.61) | 0.60 |
| | Female | 499 | (5.31) | 1.24 |
| Common pigeons | Male | 1,913 | (9.92) | 1.51 |
| | Female | 367 | (9.88) | 2.46 |

are shown in figure 4. Some slight irregularities in these curves evidently arise from the small numbers of individuals taken at certain ages. Other data in our possession show that these curves are substantially normal, and that the male ring doves—at least in mature stages—average slightly heavier than the females. In common pigeons this sex difference is present, and is indeed somewhat greater than it is in ring doves. Essentially the same data for body growth which we have tabulated here for the first time were earlier submitted to Professor S. Brody, who has already constructed and published (1927) the necessary curves for body growth of the common pigeon and the ring dove. These will be found in his study (fig. 12, p. 24). In that and other papers Brody has further compared the rate of growth of the dove and pigeon with other animals, and has noted the exceptionally high growth rate of these particular birds.

Birds seem to have relatively the highest growth rate found in the vari-

ous classes of vertebrates. Birds alone possess a bursa Fabricii. The period of bursa growth coincides precisely with the period of extremely rapid body growth. Are these associations merely and purely fortuitous?

HISTORICAL AND DISCUSSION. Kaufman (1927) has published data on the growth of the body, and of the testis, in common pigeons. The ages examined were 1, 3, 5, 7, 9, 11, 13, 15, 21 and 27 days after hatching; 3 months (3.6 months by our reckoning); and "old pigeons." At each stage 10 birds were used (no weights of ovaries). The curve obtained for body growth is quite comparable with that which could be constructed from

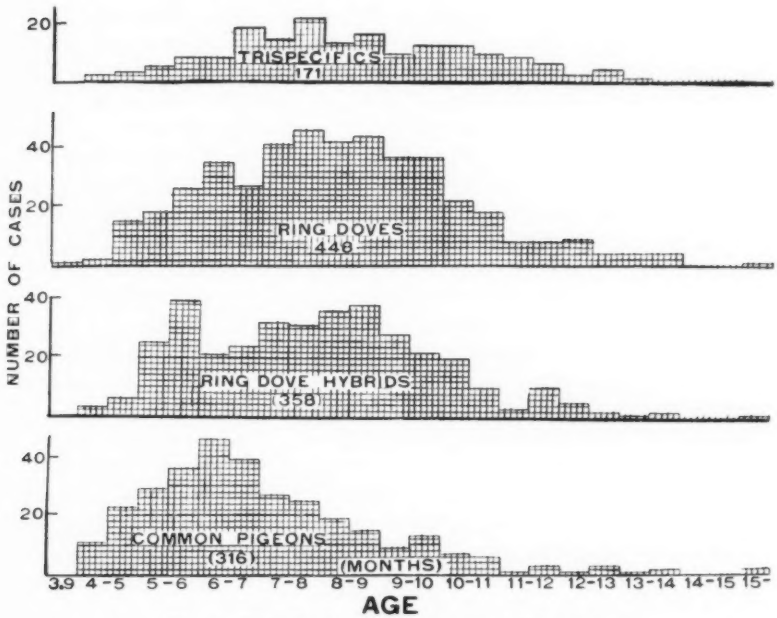



Fig. 8. Distribution of age at sexual maturity

our data for common pigeons. Testis weights were first taken on the fifth day after hatching. The data given for the testes of the young birds do not seem to have been obtained with accuracy. For example, the weight at ages 5, 7, 9 days are all given as 0.01 gram; at 11 days, as 0.03 gram; at 15 days as 0.04 gram and at 21 and 27 days, as 0.03 gram. Kaufman calls attention to the fact that the duck doubles its hatching weight in the first 6 days; the chick does the same in 9 days; while the pigeon accomplishes this in 3 days.

A few studies on growth in the common fowl provide some data con-

cerning the growth of the gonads. Latimer (1924) weighed approximately 50 ovaries and 50 pairs of testes of white leghorn fowls killed at frequent intervals between hatching and maturity. The form in which the data are published make it clear only that the ovary grows very slowly up to about 160 days. It is stated that a brief prepuberal period of rapid ovarian growth lasts but about 30 days—from about 160 to 190 days. The testes are said to have 1, "a period of slow growth to about 50 days; then 2, an increase in growth from 50 to 180 days; and then 3, a period of rapid or puberal growth from 210 to 260 days; and 4, a terminal plateau or period following maturity." Mitchell, Card and Hamilton (1926) weighed the testes (no ovaries) in a number of Plymouth rocks. They examined 9 groups, aged 29 to 324 days, in most of which 5 birds were represented. Their data indicate a very slow growth of the testis up to some (unidentified) time between 117 and 169 days. Stieve (1918) notes that the ovary of the fowl (race?) begins a more rapid growth at or soon after 4 months, and that individual follicles then for the first time begin to appear on its surface. Pezard (1919) described as normal an interruption in the body growth of pullets a few weeks prior to their commencement of laying, and found no similar interruption in 3 ovariectomised fowls. This matter is of such importance as to justify a careful and adequate repetition of Pezard's tests. In none of these studies have ovarian growth or sexual maturity been examined in relation to the growth and involution of the bursa.

Though it has long been known that the bursa Fabricii usually disappears or becomes reduced in old birds very few quantitative data are available concerning the time it attains a maximum size or the time of its disappearance. Jolly has contributed nearly all the few measurements made on birds of known age. In the most comprehensive study that has been made on this subject Jolly (1914) weighed the bursa and testes of 21 birds aged 2 to 12 months, and concluded that the highest development of the bursa is there attained at the age of four months. In the duck this is stated to occur at a similar or slightly more advanced age; while in the common pigeon involution is said to begin at 2.5 to 3.0 months (3.1 to 3.6 months by our method of reckoning age). In this important paper the temporary or accidental involution of this organ, in both fowl and pigeon, is first and fully shown. In an earlier study, Jolly (1913) had observed that the bursa usually disappears prior to sexual maturity in the fowl, duck and pigeon.

Speaking of conditions in the fowl, Jolly makes the following observations (1914, p. 455–6).:

> The maximum development of the bursa Fabricii is therefore found exactly at a stage in which the testicles are small and immature, but in which the spermatogenesis is in full preparation; the beginning of involution coincides exactly with the appearance (l'apparition) of sexual maturity. It is not unreasonable to suppose that the bursa Fabricii gives to the organism substances utilized in the sexual crisis; this is a

question which we shall study later. We are content to remark, according to figures found here, that, at the moment in which sexual maturity appears and at which the bursa involutes, the thymus is at the maximum of its development and involutes after that only very slowly and very irregularly. The involution of the bursa then clearly precedes that of the thymus.

From the above it seems that what Jolly calls the "appearance of sexual maturity" is a different and earlier thing than that which we regard as sexual maturity. The testicles of the fowls which he identifies with this period have only 3 to 4 per cent of the weight of those of his fowl aged 12 months. His tabular data show that ripe spermatozoa were first found in birds of 5 months, and indeed that only three of the six birds of this age were producing sperm. We find a long period (2 to 4 months) intervening between the beginning of bursa involution and the actual attainment of sexual maturity—as this is defined by age at the production of the first egg. Jolly gives no data for females. In doves and pigeons the testicles are both minute and immature at the beginning of bursa involution; and we have here shown that this latter period marks the beginning of a *new step toward* sexual maturity in both males and females. We believe that Jolly's data should, in fact, be similarly interpreted.

Jolly also thinks that the bursa involutes prior to the thymus. We have not studied the fowl, but incline to think that the number of cases studied at the crucial age by Jolly is quite insufficient to establish a difference. In doves and pigeons we find that they both begin involution at the same time; and that a difference lies in the circumstance that involution is complete in the one and only partial in the other. Elsewhere, Jolly has forcefully advocated the view that the thymus and bursa have the same function. "The bursa Fabricii can be considered a cloacal thymus." Our own studies on this organ incline us to agree with this conclusion. The phenomena of growth and involution in these two organs certainly seem to link them closely together. The present data, much better than the small numbers used by Jolly, show how the normal size changes in the one are similarly shown in the other. Jolly thought it not unreasonable to suppose that the bursa prepares a hormone-like substance somehow related to the sexual crisis. We think the present data supply some evidence for such a relationship. Our data show the very intimate (though not invariable) relation that exists between complete bursa involution and sexual maturity in both the pigeon and the ring dove.

The nearest our studies have come to an indication of a precise function of the bursa is found in the two demonstrations: First, that the growth of the gonads is repressed during the growth of the bursa (and thymus). Second, that the bursa normally *disappears* coincident with sexual maturity, and it *begins to involute* precisely when a new step is taken toward sexual maturity. If these relationships are real, not fortuitous, they would seem necessarily to rest upon an endocrine—not a nervous—basis.

If it transpires that the bursa and thymus have quite the same function it is important to note that no effects of thymectomy in *young* birds would appear until after the complete involution of the bursa. Also, whether the bursa would ever completely involute after complete thymectomy of a young bird is a point now wholly unknown. In other words, it is perhaps probable that a complete "thymectomy" in young birds involves the removal of both thymus and bursa.

The size difference between ovary and testis at hatching seems to us a very important matter which requires special consideration. In an earlier study (Riddle, 1927) we have considered the source of a number of sex differences—including antagonisms—in the growth *in utero* of the mammalian male and female fetus. We there also noted that some similar conditions apply to the embryos of birds, and to still lower vertebrates. The present data on gonad size at hatching constituted our evidence (not specifically cited) that growth effects are here induced by the female (follicular) hormone whose presence in the egg yolk of birds and fishes had been demonstrated by Fellner (1925). Soon after the appearance of our paper Schmalhausen (1927) published some data showing that precisely similar differences in gonad size are found in chick embryos. In all of his 11 comparisons of gonad size—from the sixth to the seventeenth day of incubation—Schmalhausen finds that the ovaries are the larger (20 to 352 per cent). In fowls, and in two families of Columbae, it is now known that during the embryonic period the ovaries are much larger than the testes. Schmalhausen studied the growth of many embryonic organs, and concerning the gonads he states as follows:

> The sex glands occupy a separate position in the series. Of course this can neither be conditioned by differentiation nor be a result of (embryonic) function. In these cases we must assume the presence of special inhibiting agencies which temporarily hold the growth of these organs within narrow limits. This is all the more probable since the growth of the testis runs an entirely distinctive course.

A further fact which would seem to throw light on this matter is the probability that the follicular hormone exercises an antagonistic or retarding action on the testes of post-natal young animals. Such effects on rats have been reported by Laqueur, Hart and De Jongh (1926), and by Steinach and Kun (1926). In young doves Riddle and Tange (mostly unpublished work) got only unfavorable effects on both testes and ovaries. From the work of others, and from other data elsewhere cited (Riddle, 1927), it seems probable that the female hormone under discussion does adversely and particularly affect the growing testis of animals in post-natal stages.

It seems to us that the several above-mentioned facts belong together, and that they probably supply the explanation for the observed size difference of ovary and testis in the bird embryo. The egg, according to Fell-

ner, is well supplied with the hormone—30 yolks from the hen containing more than a human placenta, and as much as 90 corpora lutea. This hormone is reported often to favor ovarian growth and to be adverse to testis growth in post-natal stages. The embryonic ovary and testis are subjected to what probably amounts to a continuous dosage of this hormone. Their size relations at the end of this "natural" dosage therefore apparently reflect these usual actions of this hormone.

A consideration of the relative size of testis and ovary in the mammalian fetus is, in the main, beyond the scope of this paper. If, however, our proposed explanation of conditions in the bird is to be accepted, it is necessary to consider whether that view is tenable in the light of conditions found in other groups of animals. Weights are available for the human, rat and pig, and in all these cases the fetal testis is markedly larger than the ovary, although it is probable that the placenta permits the entrance of the follicular hormone to the fetus. This would at first appear to be adverse to the above-described view. But an examination of the actual gonad weights in fetus and adult indicates, in our opinion, that repression of testis growth occurs also in these animals. In later fetal stages the testes (usually weighed with the epididymis) are only about twice the size of the ovaries; while in the adults the testes are more than four times as large as the ovaries. Further, definite signs of adverse growth effects in the fetal testis of various mammals have been described by a number of workers. I have earlier (Riddle, 1927) considered the reasons for applying to these mammalian cases the same explanation which is here applied to the bird.

If our interpretation of the origin of the size differences in the gonads of bird embryos is correct it follows that we here meet some phenomena of embryonic development which are called "normal," but which are, in the usual sense of the word, quite abnormal. Probably all that the word "normal" can ever mean in development is merely "the usual." Such modifications as are observed in these gonad size differences arise from the circumstance that the ovum—developed in a *female* organism, and a solute for both aqueous and lipoid material—is forced to accept a quantity of a maternal hormone before taking up an independent existence. It is difficult to see how such modifications differ, in any way or principle, from those resulting from specific treatments or conditions imposed by an investigator. This case should assist our thinking of all development—all heredity—as a full partnership of genetic factors and specific conditions. Many different animals can be had from a single gamete through variations of either genes or conditions. The question of "normality" scarcely enters at all. A set of genes which give a grotesque animal under one set of conditions may yield a quite balanced one by a mere change of conditions, and *vice versa*. In the field of development a gene is no less blind and adventitious than is a specific condition.

SUMMARY

The bursa Fabricii attains its maximum size at the age of 2.9 (common pigeons) and 3.1 months (ring doves); age being calculated from the beginning of embryonic development. This period of its rapid growth coincides with rapid growth of body and of thymus; and with very slow growth in the gonads.

The involution of the bursa begins, at least in early-maturing birds, immediately after its maximum size is attained. At precisely this same time the thymus begins its partial involution, and testis and ovary begin a greatly increased rate of growth. Bursa involution is usually, but not invariably, practically complete at the time sexual maturity is attained. Cases of persistent bursa are found, and their significance is quite unknown. Notable sex differences in bursa size, growth or involution are not present.

The more intimate knowledge of the bursa emphasizes its relationship to a period of extremely rapid body growth, to a period of repressed growth in the gonads, and to the time of attainment of the capacity of either sex to reproduce. These data provide some evidence that this organ has an endocrine function. Also, that the bursa is most closely associated with the thymus, and that these two organs may have an identical function.

Testis and ovary grow very slowly during the period of rapid growth in the bursa (thymus and body); they begin a much higher rate of growth closely coincident with beginning involution in the bursa (and thymus). The slow growth in testis and ovary in the early post-natal period is interpreted as modified or suppressed growth associated with growth in the bursa (and thymus).

The rate of body growth in doves and pigeons is exceptionally high as compared with that of any other bird, mammal, or other vertebrate hitherto studied. Birds alone of these groups possess a bursa Fabricii.

Data are given for the age at which sexual maturity is attained in ring doves and in common pigeons.

An unexpected disparity of size is found in the testes and ovaries at hatching—the ovary being about twice as large as the testes, although the adult testis is 3 to 5 times as large as the adult (resting) ovary. This is interpreted as a result of the presence of the female or follicular hormone in the egg, and of its specific action upon the embryonic gonads.

This modification of the embryonic growth of organs as the result of the regular, though essentially accidental, inclusion of a maternal hormone within the egg is of interest in the theory of development or heredity. The conditions actually found are currently considered normal, though they are quite as abnormal as those effected by the experimentalist. In development, the word "normal" can merely mean "the usual."

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# STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

## XXIV. On the Extirpation of the Bursa Fabricii in Young Doves

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Received for publication June 14, 1928

No studies involving the removal of the bursa Fabricii have been reported. Other studies made in this laboratory supply some evidence that the bursa, which is found in birds only, has an endocrine function. To us its function appears to be in some way associated with growth and reproduction (Riddle, 1928), and it was thought that observations on these processes in birds deprived of the bursa should supply further light on this problem. As a descriptive term for the process of extirpation of this bursa, we shall introduce and use the word "bursectomy."

During the time that has elapsed since these operations were performed in 1925 it has become increasingly probable that the bursa and the thymus each, at least in part, duplicates the function of the other. If this proves to be true it is evident that bursectomy alone, or thymectomy alone in the case of young birds, may quite fail to supply evidence of the kind we most desire. We have not performed these two operations on the same bird. Nevertheless, it is thought that the further investigation of the problem will be advanced by a brief account of these results—particularly since these are the only known records of bursectomy.

At the outset it is necessary to note the things which are directly and indirectly involved in the removal of the bursa. The organ is easily accessible and a local anesthetic only need be used. A horizontal slit is made through the skin and body wall just above the anus. This permits a curved-tip forceps to be passed over the dorsal aspect of the bursa and grasp the latter near its anterior end; with another forceps much of the length of the bursa is freed from the light adhesions of its ventral surface with the dorsal wall of the cloaca. The bursa, and the point of its union with the cloaca, can then both be drawn nearly or quite outside the body. The bursa, however, is broadly united with the posterior dorsal wall of the cloaca; *and this point of attachment is in very close proximity to all of the ducts of the entire genito-urinary system.* The bursa—being in fact an evagination of the cloacal wall—must be cut extremely close to the cloaca if it is to be removed completely. This procedure, together with

the stretching involved in getting the bursa into position to remove, renders it very difficult to avoid tearing or cutting also one or both ureters, one or both sperm ducts, or (in the case of females) one or both of the oviducts (two are present in very young birds). In any considerable series of bursectomies some or all of these undesirable things will occur. The cutting of the sexual ducts may of course have important effects on the bird's later capacity to reproduce—and the relation of uncomplicated bursa extirpation to reproduction was the thing particularly sought in this study. The section or laceration of one or both ureters may of course decide the bird's chance to live. The cutting of the cloaca was usually followed by an acute peritonitis, fatal within a few hours.

DESCRIPTION OF RESULTS. The study was carried out on young ring doves (*Streptopelia risoria*) and on ring dove hybrids. A small number of preliminary bursectomies was made by the senior author in 1922. From 5 male and 5 female doves the bursa was removed at ages ranging from 6 to 33 days (2 at 66 days) after hatching. In this preliminary study no later exploratory operations were made to test the completeness of the original operation, or to search for regenerating bursa tissue. One of the females died at 4 months; the other 9 birds were reared to maturity. All these birds appeared to grow normally and to attain their normal adult body weight (♂♂ 161; ♀♀ 153 grams). They were killed at ages ranging from 9.6 to 14.9 months.

The four females *a*, all showed persistent right oviducts; *b*, two had persistent right ovaries; *c*, in 1 the right kidney was completely absent; and *d*, all produced their first egg at a rather advanced age (9.4 to 12.6 months). Of the 5 males 2, or perhaps 3, had sperm ducts which were nearly or quite normal. One had a very large left (3 times right) sperm duct which was filled with fluid and was probably without opening to the cloaca. The corresponding (left) epididymis was much larger (50.3 mgm.) than the right (21.6 mgm.). In another male the right sperm duct was similarly distended throughout its length, and its normal outlet to the cloaca was probably occluded; the left duct here seemed inadequately developed. The fifth male had a fairly well developed left Müllerian duct (oviduct) 38.5 mm. long, extending from cloaca to ovary. This was filled with a gelatinous fluid; 10 mm. from its anterior end it carried a small nodule 2.6 × 2.0 mm.) of unrecognized nature. The sperm ducts here were apparently normal. These results, and other considerations, supplied further reason for extending this study to a larger number of individuals.

In our later series of tests 19 males and 37 females were first operated at ages ranging from 5 to 18 days after hatching (20 to 37 days as we calculate age). Partly because of our special effort to remove all traces of bursa tissue the cloaca was cut, or other injury was inflicted, and 21 of

the 56 birds died at from 1 to 25 days after the first operation. Eight died 27 to 114 days after operation; and one female was killed at the age of 114 days for a complete examination of the incretory organs. Fourteen re-bursectomies, or exploratory operations, were done at 16 to 97 days after the first operation. Among these reoperated birds 4 died after 1 to 13 days. Eight males and 14 females were reared to maturity and individual records for their body growth and reproductive characteristics were obtained.

The body weights of the operated birds were taken at 10-day intervals from the time they were 30 days old to the age of 180 days. Figure 1 gives point-to-point growth curves constructed from the mean body weight of the males and females. This is the solid line of the figure. The dotted line gives the normal growth curves of unoperated ring doves, similarly calculated from data supplied elsewhere by Riddle (1928). Inspection of the individual records shows that the operation was always followed by a temporary check in growth which sometimes lasted several days; the somewhat lower weights of the operated birds at 40 days are certainly thus explained. It seems clear that bursectomy, in those birds which were reared to maturity, produced no real change in the rate or amount of body growth.

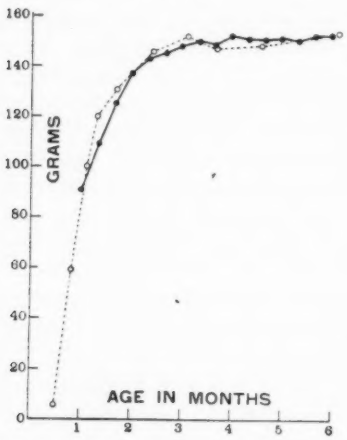


Fig. 1. Growth curve of bursectomized ring doves (solid line). Growth curve of unoperated ring doves (dotted line).

Sexual maturity seemed to be attained at rather advanced ages in the four females of our preliminary series. It was very desirable to test this point adequately in a larger group of birds. In these latter tests the sisters of the operated birds were bred, and the age at which they produced their first egg was noted. These sisters supply a better test than do the mothers of the operated birds, since birds for operation were here selected from mothers which had not matured at a very advanced age. The results are shown in table 1. The 14 operated females show an average age of maturity of 8.0 months; their sisters, 7.9+ months. We therefore find no effect of bursectomy on the age at which female birds became sexually mature.

Other phenomena connected with these bursectomies, but wholly or in part resulting from the section of genito-urinary ducts, are various in

kind and difficult to describe. We have attempted to give the most essential and significant findings in tabular form. The data of table 2 show that nearly all of the unusual or abnormal conditions already described as present in the few birds of the preliminary series reappear in the later and larger series. That we sometimes accomplished the complete removal of the bursa at the first operation is attested by 3 biopsies of the table (4 in a total of 14); in the 7 additional tabulated examinations of this sort

TABLE 1

*Age of maturity in bursectomized female doves compared with their mothers and sisters*

| NUMBER OF BIRD | AGE (MONTHS) AT MATURITY OF | | | |
|---|---|---|---|---|
| | Bursectomized | Mother | Sisters | |
| | | | Individually | Average |
| 1 | 11.4 | 7.9 | 12.1, 11.2, 8.8, 8.0 | 10.0 |
| 2 | 10.1 | 8.7 | 7.2 | 7.2 |
| 3 | 9.8 | 5.4 | 10.8*, 12.1* | 11.5*+ |
| 4 | 8.7 | 5.6 | 10.0, 9.8, 8.3, 6.6, 5.6 | 8.1 |
| 5 | 8.6 | 9.4 | | |
| 6 | 7.5 | 7.4 | 8.3, 5.8 | 7.1 |
| 7 | 7.6 | 7.5 | 8.3, 5.8 | 7.1 |
| 8 | 7.6 | 5.1 | 6.5 | 6.5 |
| 9 | 6.5 | 5.1 | | |
| 10 | 7.2 | 8.7 | 7.2 | 7.2 |
| 11 | 6.8 | 7.4 | 8.3, 5.8, | 7.1 |
| 12 | 6.3 | 7.6 | 6.7, 5.5 | 6.1 |
| 13 | 7.2 | 7.6 | 6.7, 5.5 | 6.1 |
| 14 | 6.7 | 7.2 | 10.6 | 10.6 |
| Average... | 8.0 | 7.2 | | 7.9+ |
| Preliminary series | | | | |
| A | 11.0 | 9.9 | | |
| B | 13.5 | 9.9 | | |
| C | 14.9 | 5.2 | | |
| D | 11.7 | 5.2 | | |

* Still immature when killed at this age.

only 5 to 36 milligrams of bursa tissue were found and removed. These small amounts of bursa tissue, taken from healthy birds at ages when the normal bursa would be quite large, make it evident that the bursa has little or no power of regeneration under the conditions observed in our tests.

In the females of the later and larger series the right oviduct is present in no larger proportion than is found among unoperated birds. The ab-

TABLE 2

*Special data on bursectomized birds raised to maturity*

| NUMBER OF BIRD | AGE | | | RIGHT OVIDUCT PRESENT | RIGHT OVARY (♀ ♀) SPERM DUCTS (♂ ♂) | REMARKS |
|---|---|---|---|---|---|---|
| | Operated | Reoperated | Killed (or dead) | | | |
| **Females** | | | | | | |
| | *days* | *days* | *months* | | | |
| A | 39 | | 11.0 | Posterior part | 36 mgm. | |
| B | 48 | | 13.5 | Full length | None | Right kidney absent |
| C | 81 | | 14.9 | Posterior and middle | 20 mgm. | |
| D | 22 | | 11.7 | Posterior and middle | None | |
| 1 | 25 | | 18.8 | None | None | |
| 2 | 25 | 48 | 18.9 | None | 1 ovum only | |
| 3 | 30 | | 9.8 | Posterior and middle | 11 mgm. | Right kidney absent |
| 4 | 26 | | 11.8 | None | Traces | |
| 5 | 30 | | 13.7 | None | 4 small ova | Dead |
| 6 | 33 | 56 | 17.9 | None | None | Diseased |
| 7 | 31 | 54 | 13.8 | None | Traces | |
| 8 | 32 | | 9.8 | None | 8 mgm. | Dead |
| 9 | 30 | | 12.9 | None | Traces | |
| 10 | 23 | 88* | 11.0 | None | Traces | |
| 11 | 26 | | 9.6 | Posterior part | Traces | |
| 12 | 29 | | 10.6 | None | 5 mgm. | Diseased |
| 13 | 27 | | 15.5 | None | None | |
| 14 | 31 | 91 | 22.4 | None | 1 ovum only | |
| **Males** | | | | | | |
| A | 45 | | 11.1 | Full length (left) | Normal | |
| B | 42 | | 11.1 | | Normal | |
| C | 20 | | 9.6 | | Left large, closed | Left epididymis enlarged |
| D | 21 | | 10.4 | | Normal | |
| E | 81 | | 12.4 | | Right large; left small | |
| 1 | 27 | 49 | 12.5 | | Left much smaller | |
| 2 | 26 | | 18.2 | | Normal | |
| 3 | 30 | 101* | 19.0 | | Left occluded, large | |
| 4 | 33 | 101 | 13.1 | | Normal (?) | Diseased |
| 5 | 23 | 37 | 10.7 | Partial (left) | Normal (?) | |
| 6 | 27 | 100* | 12.5 | | Normal | |
| 7 | 27 | | 11.1 | | Normal (?) | Testes minute |
| 8 | 27 | | 8.5 | | Normal | |

* No bursa tissue discoverable.

sence of a right oviduct in the four dead or diseased females, however, is by no means as significant as its absence in healthy birds; among these latter it was present in 2, absent in 8. But all of the four females of the preliminary series had this right oviduct, and a total of two males showed a quite abnormal development of the *left* oviduct. Possibly, but we can not say certainly, the right ovary persisted in an abnormally high proportion of cases—in 13 of 18 tests. The expected proportion of persistent right ovaries in these ring doves seems to be changing in our material. Several years ago we noted the presence of the right ovary in 47 of 200 healthy ring doves. A count made for the 356 females most recently autopsied by us shows as many birds with right ovaries (177) as without them (179). If these ovaries and oviducts are, in fact, present in unexpected numbers in the operated animals this is a matter which it is difficult to trace to indirect effects of bursa removal.

In addition to the tabulated data we obtained complete records on many aspects of reproduction in these operated birds. Reproduction in the females was accompanied by numerous abnormalities and irregularities. But these probably resulted not from the loss of the bursa but from section of the sexual ducts. Among the 14 females of the later series, 9 produced some unpaired eggs; 5 produced some stringy, albuminous, shell-less, yolk-less, masses; 5 threw some soft-shelled eggs. Only 7 of the 14 birds produced 1 or more hatchable eggs; in only 1 case did either of the very first pair of eggs laid by the bird show any fertility. One female (no. 3) was killed by us when she proved unable to pass the first egg in life through a blind or closed oviduct. From the 165 ovulations recorded for these birds only 39 embryos could be hatched; 51 properly formed eggs proved infertile. The operated males, mated to unoperated females, had somewhat better fertility records. From 68 tests there were 43 fertilizations (36 hatched) and 25 failures. Two of the eight males fertilized no egg in 14 tests. One male (no. 3) fertilized 15 eggs in 15 tests. The testes of all the males, with one exception, were probably of normal size. The size of the ovaries of the bursectomized birds also falls within the normal range of variation of this organ.

Discussion. Our description has implied the complete disappearance of two kidneys as a sequel to the section or tearing of the corresponding ureters. Reasons for this interpretation should be given. First, a few thousands of unoperated ring doves such as were used in this study have failed to supply a single case of an absent kidney.[1] Two small series of bursectomies each supplied such a case. These bursectomies are the only operations that we have performed on these birds that would probably

[1] Two cases of unpaired kidneys have indeed been observed in another genus (*Turtur*) of pigeons; but in both of these cases the bird showed also other gross abnormalities, and neither was ever mistaken for a normal bird.

involve a section or closure of the ureters. Second, the death of several of the birds at periods variously removed from the date of operation gave macroscopic evidence of kidney disease. For example, the first autopsy of a bursectomized bird, dead 93 days after operation and aged 4 months, was set down as due to kidney disease. Third, various considerations oppose the view that the loss of the bursa was directly responsible for the disappearance of kidneys. We wish only to emphasize that in all probability these cases were not of embryonic or congenital nature, and that the pathologist will find their origin accessible to study.

It is well to point out that right ovaries and oviducts are usually not present, and when present they are uncommonly small, in doves and in most other birds. But an examination of a large amount of control material leaves it doubtful whether the high proportion of persistent right ovaries and oviducts in the bursectomized birds is other than accidental. If they are not accidental these phenomena are much more difficult to regard as indirect effects proceeding from the section of urino-genital ducts than are the other abnormalities noted. They seem to go with individuals in which the feminine is accentuated. We think that, without doubt, the two cases of left oviducts present in males must be regarded in the same way. One of us has observed the rare occurrence of oviducts in males as an intersexual character. Male doves with an oviduct have usually brought with them other evidence of feminine leanings. And in practically all cases it is the *left* duct only that is present—the same one that is well developed and functional in the female. These two oviducts, found among the 13 bursectomized males, provide the best—perhaps the only—immediate evidence that the removal of the bursa may somewhat affect sexuality.

Though our bursectomies involved the complications that have been noted they have made it quite clear that removal of the bursa at 10 to 20 days after hatching does not modify the body growth of ring doves. They also make it clear that females deprived of bursa tissue attain sexual maturity at the usual age. These results would follow not only in case the bursa has no relation to these phenomena, but also in case the bursa and thymus have a similar relation to growth and sexual maturity.

The lack of regeneration of bursa tissue is in striking contrast to the free regeneration found in the *adult* thymus by Riddle (1924). The following question arises: If bursa and thymus have a common function, and nearly all of the bursa tissue is removed during its period of growth should we expect hypertrophy of the thymus, or shall we get regeneration of the bursa? We actually got little or no regeneration of the bursa; no search was made for possible changes in the thymus. It would seem that further knowledge of the functions of both bursa and thymus can best be obtained by the complete extirpation of both of these organs in young

birds, and by the later study of growth and reproduction in such animals. It is thought that the present inadequate study will prove helpful in that rather difficult task. If the bursa Fabricii should prove to be a "cloacal thymus," as maintained by Jolly (1914) and suspected by Riddle (1928), this result in birds may throw doubt upon the assumption that the thymus is the only organ having thymic function in mammals. And, the significance hitherto attached to the negative results obtained on thymectomized mammals would be greatly minimized.

## SUMMARY

The bursa Fabricii was removed from 66 ring doves at a few days or weeks after hatching. Biopsies or re-operations were later performed on 14 of these birds. The mortality was high and only 31 birds were reared to maturity.

Bursectomy does not change the rate of growth nor the normal adult body weight of those birds which live to maturity.

Bursectomy has no effect upon the age at which female birds become sexually mature.

These results would follow if the bursa has no function whatever; but they are also consistent with the view that the functions of the bursa, in part at least, are duplicated in the thymus. Only removal of both thymus and bursa in birds will serve adequately to demonstrate the functions of either organ.

It is probable that the removal of the bursa was responsible for the development of left oviducts in 2 of the 13 operated males. Possibly more than the expected number of right ovaries and right oviducts were present in the operated females as a result of the removal of the bursa.

The extirpation of the bursa, as done by us, involves the occasional section of ureters, oviducts and sperm ducts. These undesired accompaniments of the operation resulted in numerous irregularities and abnormalities of reproduction. The section of the ureters was probably responsible for many deaths 5 days to 3 months after operation; and they probably caused the complete disappearance of the right kidney in two operated animals.

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# THE EFFECT OF INCREASED BLOOD FLOW ON THE RATIO BETWEEN OXYGEN CONSUMPTION AND PULMONARY VENTILATION

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Received for publication June 11, 1928

In the lung, as in nearly every organ of the body, there exists a large margin of functional reserve which may be called into play when conditions demand. It has been repeatedly demonstrated that large areas of the vascular bed of the lungs can be excluded from the circulation without apparent influence on the blood pressure in the systemic circuit or on the output of the left ventricle. Similarly, recent studies in the measurement of cardiac output in rest and exercise have shown that the pulmonary circuit as a whole may suddenly be called upon to handle a four-fold increase in volume flow per minute (1). The obvious response to either of these demands for an increased oxygenation of blood is an increase in the respiratory minute volume brought about by an alteration of the rate and depth of breathing. In addition, however, there may be a further compensatory mechanism in the form of an actual increase in the effective diffusing surface. Undoubtedly such a mechanism would tend to be closely linked with the alteration in breathing.

This paper is concerned with the experimental demonstration of the fact that, under the conditions imposed by our experiments, an increase in the volume blood flow in the lung is accompanied by an increase in the ability of the lung to absorb oxygen; and that such an increase in the effectiveness of pulmonary ventilation may occur in a naturally breathing animal as a phenomenon totally independent of alterations in the respiratory minute volume, and the rate or depth of respiration.

METHOD. A cat is completely anesthetized by an intravenous injection of sodium barbital (5 per cent solution) and prepared in the manner described by Churchill and Agassiz (2), which permits the separation of the air breathed by the two lungs. In brief, this is accomplished by tying the end of a long cannula in the left primary bronchus. An air-tight closure of the chest is then made by suturing the pericardium to the edges of the window made in the anterior wall of the chest by the removal of the sternum. In order to secure a continuous record of the oxygen consumption and the respiratory minute-volume, the air-way leading from each

lung is connected with a closed spirometer system consisting of a tube of soda-lime and a Brodie bellows,—the latter writing directly on the kymograph (fig. 1). The animal breathes an atmosphere of pure oxygen as in the familiar graphic metabolism apparatus. A similar modification of the original method has been employed by Moore (3).

In order to increase the functional demand on one lung, the branch of the pulmonary artery to the opposite side is suddenly occluded by compression with a ligature previously placed about the vessel. This shunts the entire output of the right ventricle into one lung, thereby more than doubling the volume flow in the left lung when the right pulmonary artery is shut off. A similar diversion of the volume flow may be produced by occluding either set of pulmonary veins. The placement of the ligatures

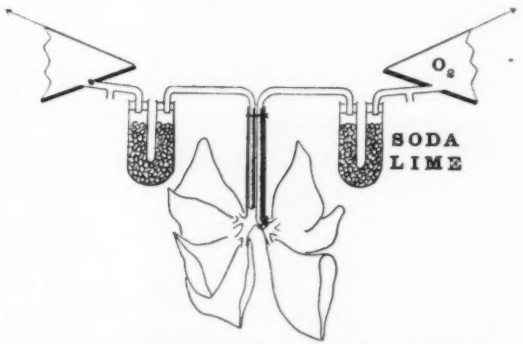


**Fig. 1. Diagrammatic sketch of apparatus. The two cannulas are shown in the trachea, the one leading to the left lung being anchored in place by a ligature around the left primary bronchus. The two spirometers are Brodie bellows writing directly on the drum.**

about these vessels within the pericardium is made relatively simple by the exposure of the heart which this preparation affords. Occlusion of the right pulmonary veins, however, must be accomplished by a ligature placed at the level of the hilum of the lung, outside of the pericardium. Although an intrapericardial ligature can be placed about these vessels, traction upon it is followed by a marked depressor reaction in the systemic circuit with a diminution in volume blood flow. This reaction was commented upon previously (2), and is now thought to be due to the stimulation of nerve endings at the base of the heart. It can be avoided by placing the ligature outside the pericardium as stated above.

A copy of the protocol with a section of the kymograph tracing is given for a typical experiment. In the other experiments, some of the numerical results of which are presented, the procedure was essentially the same. In

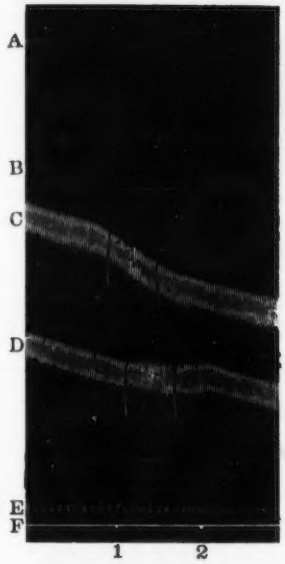


Fig. 2

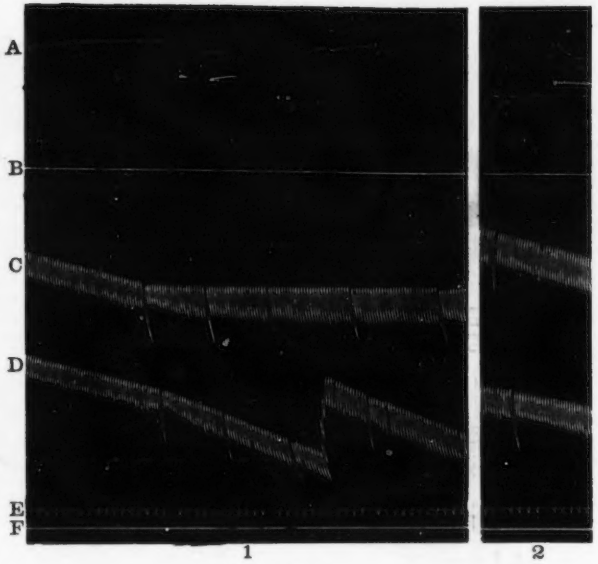


Fig. 3

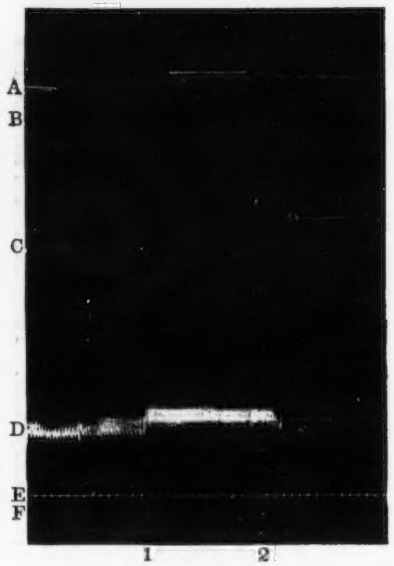


Fig. 4

Fig. 2. Kymograph record of experiment 1. A: systemic blood pressure in millimeters of mercury measured in right carotid artery; B: zero line for blood pressure; C: spirometer tracing of left lung, down stroke of lever during inspiration; D: spirometer tracing of right lung; E: time in 5 second intervals; F: base line for determination of oxygen consumption and signal magnet. At the first mark the right branch of the pulmonary artery was occluded; at the second mark it was released.

Fig. 3. Kymograph record of experiment 1. Tracings as in figure 2. At the signal mark in section 1, the left pulmonary veins were occluded. The upward shift of the tracing of the right lung indicates a refilling of the spirometer with oxygen. Section 2 is shortly after the release of the ligature around the veins, showing the return to normal relationships.

Fig. 4. Kymograph record of an experiment in which pulmonary artery pressure was recorded. A: systemic blood pressure in the carotid artery. No zero line is recorded, the height as computed by suitable correction from the baseline, being 90 mm. of mercury at the left margin of the tracing. B and C: tracings of the respiratory movements of the left and right lungs respectively. D: pressure in main stem of pulmonary artery recorded in centimeters of water. Mean pressure was 18 cm. before, and 23.5 cm. during occlusion, immediately afterwards falling to 16.5 cm. E: time in 5 second intervals; F: base line and signal magnet. The latter records at points 1 and 2 the occlusion and release respectively, of the right branch of the pulmonary artery.

two experiments a record was made of the pressure in the pulmonary artery; a section of the kymograph tracing of one of these is reproduced in figure 3. The pressure in the pulmonary artery was obtained by inserting a cannula (4) in the main stem of the artery. This was connected with a small recording tambour on the far side of which was placed a water manometer. The addition of the water manometer not only provides a direct reading of the pressure, but also permits a to and fro movement of heparinized blood in the tip of the cannula which helps to prevent clotting.

*Experiment 1.* December 15, 1927. Cat, weight 3.7 kgm. Divided breathing preparation. Systemic blood pressure in carotid artery. Occlusion and release of right branch of pulmonary artery.

| | |
|---|---|
| 2:05 | Induction of anesthesia by chloroform and ether to permit injection of 20 cc. 5 per cent solution barbital sodium in the left femoral vein |
| 2:15 | Complete anesthesia with barbital-sodium |
| 2:30 | Artificial ventilation started for period during which chest is opened |
| 3:10 | Artificial ventilation discontinued. Chest closed without leakage |
| 3:25 | Observations started |
| 3:30 | Right pulmonary artery occluded (fig. 2) |
| 3:30¾ | Right pulmonary artery released |
| 3:37½ | Right pulmonary artery occluded |
| 3:38½ | Right pulmonary artery released |
| 3:44 | Left pulmonary vein occluded (fig. 3) |
| 3:52 | Left pulmonary vein released |
| 3:57 | Left pulmonary vein occluded |
| 4:01 | Left pulmonary vein released |
| 4:06 | Animal killed by sectioning pulmonary artery |

*Autopsy Notes:* No air in chest. Preparation technically perfect throughout the experiment. Lungs expand symmetrically with low air pressure. No signs of atelectasis or edema.

The numerical values for this experiment are included with those of other experiments in the accompanying tables.

OBSERVATIONS. Occlusion of the right pulmonary artery was performed fifteen times in six different animals. The respiration of the left lung measured in cubic centimeters per minute was increased thirteen times, by amounts which varied from 12 to 232 cc.; remained unchanged once; and was once actually decreased by 85 cc. The oxygen absorbed by the left lung was invariably increased, from 2.5 cc. to 15 cc. per minute. When the minute volume was unchanged by the procedure, the oxygen absorption was nevertheless increased by 13.8 cc. (Dec. 15, R. P. A. O. 1); and on the occasion when the minute volume was reduced 85 cc. (Dec. 5, R. P. A. O. 2), the oxygen absorption from that lung was actually increased by 8.6 cc.

Occlusion of the left or right pulmonary veins was done seven times in four different animals. The respiratory volume of the contralateral lung was invariably increased—from 49 cc. to 207 cc. per minute. The rate of oxygen absorption in the circulated lung was also constantly increased.

afterwards falling to 16.5 cm. E: time in 5 second intervals; F: base line and signal magnet. The latter records at points 1 and 2 the occlusion and release respectively, of the right branch of the pulmonary artery.

In order to more readily compare the minute volume respiration with the rate of oxygen absorption, a decimal figure has been computed to

TABLE 1

*Right pulmonary artery occlusion*

(Volumes in cubic centimeters without temperature or vapor tension correction)

| DATE | PERIOD | RESPIRATION RATE PER MINUTE | VOLUME PER BREATH | | MINUTE VOLUME | | TOTAL MINUTE VOLUME RESPIRATION | OXYGEN ABSORPTION | | TOTAL OXYGEN ABSORPTION | OXYGEN ABSORPTION / MINUTE VOLUME | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Left | Right | Left | Right | | Left | Right | | Left | Right |
| December 3, 1927 | Control | 25 | 11.6 | 12.9 | 290 | 323 | 613 | 6.0 | 8.0 | 14.0 | 0.021 | 0.024 |
| | R. P. A. O. 1 | 26 | 13.3 | 13.4 | 346 | 349 | 695 | 11.0 | 0 | 11.0 | 0.032 | 0 |
| | Control | 25 | 12.6 | 13.3 | 314 | 333 | 647 | 6.5 | 7.5 | 14.0 | 0.021 | 0.022 |
| | R. P. A. O. 2 | 23 | 15.0 | 9.7 | 345 | 224 | 569 | 9.5 | 0 | 9.5 | 0.027 | 0 |
| | Control | 25 | 12.7 | 14.8 | 318 | 371 | 689 | 6.5 | 7.0 | 13.5 | 0.020 | 0.018 |
| December 5, 1927 | Control | 21 | 25.0 | 23.6 | 525 | 497 | 1,022 | 16.0 | 11.5 | 27.5 | 0.030 | 0.023 |
| | R. P. A. O. 1 | 22 | 25.7 | 27.5 | 566 | 605 | 1,171 | 26.0 | 0 | 26.0 | 0.045 | 0 |
| | Control | 24 | 30.9 | 24.0 | 742 | 576 | 1,318 | 18.0 | 12.0 | 30.0 | 0.024 | 0.020 |
| | R. P. A. O. 2 | 23 | 28.5 | 24.3 | 657 | 558 | 1,215 | 26.6 | 0 | 26.6 | 0.041 | 0 |
| | Control* | 20 | 26.9 | 25.5 | 539 | 510 | 1,049 | 17.0 | 13.0 | 30.0 | 0.031 | 0.025 |
| December 14, 1927 | Control | 25 | 14.3 | 16.9 | 359 | 423 | 782 | 12.5 | 13.5 | 26.0 | 0.034 | 0.032 |
| | R. P. A. O. 1 | 32 | 18.4 | 23.1 | 591 | 741 | 1,332 | 27.0 | 3.0 | 30.0 | 0.045 | 0.004 |
| | Control | 23 | 17.6 | 19.0 | 407 | 441 | 848 | 14.5 | 11.5 | 26.0 | 0.035 | 0.026 |
| December 15, 1927 | Control | 45 | 17.5 | 18.5 | 788 | 835 | 1,623 | 15.0 | 21.0 | 36.0 | 0.018 | 0.024 |
| | R. P. A. O. 1 | 44 | 17.9 | 19.9 | 788 | 875 | 1,663 | 28.8 | 3.6 | 32.4 | 0.036 | 0.004 |
| | Control | 45 | 18.9 | 19.1 | 853 | 859 | 1,712 | 16.8 | 18.6 | 35.4 | 0.019 | 0.021 |
| | Control | 41 | 13.6 | 18.6 | 558 | 766 | 1,324 | 13.0 | 16.0 | 29.0 | 0.023 | 0.020 |
| | R. P. A. O. 2 | 40 | 18.9 | 21.7 | 756 | 869 | 1,625 | 28.0 | 0 | 28.0 | 0.037 | 0 |
| | Control | 38 | 17.9 | 18.7 | 683 | 711 | 1,394 | 14.0 | 15.0 | 29.0 | 0.020 | 0.021 |
| April 12, 1928 | Control | 31 | 23.0 | 27.0 | 706 | 851 | 1,557 | 12.5 | 14.0 | 26.5 | 0.017 | 0.016 |
| | R. P. A. O. 1 | 30 | 25.0 | 30.0 | 738 | 904 | 1,642 | 27.5 | 2.5 | 30.0 | 0.037 | 0.002 |
| | Control | 29 | 27.0 | 33.0 | 784 | 963 | 1,747 | 14.0 | 15.0 | 29.0 | 0.017 | 0.015 |
| April 17, 1928 | Control | 18 | 16.0 | 13.0 | 288 | 237 | 525 | 12.0 | 11.0 | 23.0 | 0.041 | 0.046 |
| | R. P. A. O. 1 | 21 | 17.0 | 14.0 | 356 | 302 | 658 | 22.0 | 0 | 22.0 | 0.061 | 0 |
| | Control | 22 | 17.0 | 13.0 | 364 | 286 | 650 | 12.5 | 11.5 | 24.0 | 0.034 | 0.040 |

* Note: Following this, both vagal nerves were cut; under these conditions maximum volume per breath = 48 cc., (right) and 45 cc. (left).

express their numerical relationship. This in a way indicates the ability of the lung to absorb oxygen per unit of ventilation, under the varying

TABLE 2

*Right and left pulmonary vein occlusion*

(Volumes in cubic centimeters without temperature or vapor tension correction)

| DATE | PERIOD | RESPIRATION RATE PER MINUTE | VOLUME PER BREATH | | MINUTE VOLUME | | TOTAL MINUTE VOLUME RESPIRATION | OXYGEN ABSORPTION | | TOTAL OXYGEN ABSORPTION | OXYGEN ABSORPTION / MINUTE VOLUME | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Left | Right | Left | Right | | Left | Right | | Left | Right |
| December 15, 1927 | Control | 38 | 16.0 | 15.8 | 609 | 600 | 1,209 | 15.0 | 16.0 | 31.0 | 0.024 | 0.026 |
| | L. P. V. O. 1 | 33 | 23.2 | 23.9 | 768 | 789 | 1,557 | 0 | 28.0 | 28.0 | 0 | 0.035 |
| | Control | 40 | 18.0 | 19.8 | 723 | 794 | 1,517 | 15.0 | 15.0 | 30.0 | 0.020 | 0.018 |
| | L. P. V. O. 2 | 30 | 23.5 | 29.8 | 707 | 896 | 1,603 | 0 | 29.0 | 29.0 | 0 | 0.032 |
| | Control | 35 | 23.4 | 22.7 | 821 | 795 | 1,616 | 13.0 | 16.0 | 29.0 | 0.015 | 0.020 |
| January 13, 1928 | Control | 23 | 13.1 | 17.8 | 301 | 410 | 711 | 7.5 | 10.0 | 17.5 | 0.024 | 0.024 |
| | R. P. V. O. 1 | 28 | 16.8 | 18.1 | 471 | 509 | 980 | 17.0 | 0 | 17.0 | 0.036 | 0 |
| | Control | 22 | 13.7 | 18.3 | 303 | 404 | 707 | 7.5 | 10.0 | 17.5 | 0.024 | 0.024 |

TABLE 3

*The ratio between oxygen consumption and pulmonary ventilation at the beginning and end of experiments involving two or more occlusions of a branch of the pulmonary artery, as contrasted with those in which similar procedures were carried out on the pulmonary veins*

| EXPERIMENT | TOTAL OXYGEN ABSORPTION / TOTAL MINUTE VOLUME RESPIRATION | | |
|---|---|---|---|
| | Start | End | Difference |
| **Pulmonary artery occlusion** | | | |
| Cat 1 | 0.022 | 0.024 | +0.002 |
| Cat 2 | 0.026 | 0.028 | +0.002 |
| Cat 3 | 0.033 | 0.030 | −0.003 |
| Cat 4 | 0.022 | 0.020 | −0.002 |
| Cat 5 | 0.043 | 0.026 | −0.017 |
| Cat 6 | 0.016 | 0.015 | −0.001 |
| **Pulmonary vein occlusion** | | | |
| Cat 7 | 0.025 | 0.017 | −0.008 |
| Cat 8 | 0.035 | 0.024 | −0.011 |
| Cat 9 | 0.023 | 0.015 | −0.008 |
| Cat 10 | 0.023 | 0.037 | +0.014 |
| Cat 11 | 0.033 | 0.026 | −0.007 |

experimental conditions. An increase in this value indicates that the animal moves less air to obtain the equivalent amount of oxygen. With certain assumptions this value may be considered to indicate the effectiveness of the diffusing surface. Thus, when the left lung absorbs 15 cc. of oxygen at a minute volume respiration of 788 cc., the effectiveness of its efforts may be expressed by the figure 0.018. When the circulation in this lung is more than doubled by diverting the entire output of the right ventricle into it, 28.8 cc. of oxygen are absorbed with an unchanged minute volume. This ratio gives the decimal figure 0.036, indicating an increased degree of effectiveness in the lung as an oxygen absorbing mechanism. The decimal fraction so computed for the lung in which the circulation is experimentally increased, is constantly increased by the procedure.

It is to be noted that the volume per breath was constantly increased throughout the experiments, while the respiratory rate was frequently unchanged. In no case did the animal assume a rapid shallow type of respiration which would tend to increase the total ventilation but leave the effective alveolar ventilation unaltered because of the dead space.

The effect of the experiment upon the individual animals is summarized in table 3, where the ratio between the total oxygen absorption to the total minute volume of respiration is given for the beginning and the end of the different experiments. It is seen that the experimental procedure, involving two or more periods of occlusion of one branch of the pulmonary artery has no constant effect on this value. In contrast, however, the procedures involving periods of temporary obstruction of the pulmonary veins are, with one exception, followed by a decrease in the decimal fraction, indicating that the animal is moving more air to obtain the necessary oxygen. This is in accord with other generally known facts concerning the deleterious effects on the lung produced by pulmonary vein obstruction.

Discussion. At least two reservations are to be held in mind in referring data obtained from these experiments to the condition of affairs in an intact animal. First, the placing of the ligature about the left primary bronchus may be attended by trauma of unknown degree to the vagal plexus; and, further, the tying of the ligature around the bronchus onto the brass tube which is inserted into its lumen may well act to interrupt the many nerve pathways coursing along the bronchial walls. As a result, the left lung may be partially or totally denervated. Secondly, the breathing of oxygen-enriched atmosphere—a condition imposed by the experimental method—may exaggerate the possibilities of increasing the functional diffusing surface, because it enables the blood to become saturated with oxygen before it traverses but a fraction of the capillary bed. An increase in the rate of flow under such conditions may be met by the extension of the process of oxygenation throughout the entire diffusing area. In our hands the occlusion of one branch of the pulmonary

artery in an otherwise intact animal is invariably followed by an increase of the respiratory minute volume unless the inspired air be substantially enriched with oxygen.

The increase in the ability of the lung to absorb oxygen which we have demonstrated, could be accomplished by an increase in the functional diffusing surface brought about by the following methods, acting singly or in combination:

1. As noted above, if at the original rate of flow the gaseous exchange in the blood has traversed but a fraction of the capillary length which may function as a diffusing surface, an increase in the rate of flow may be met by extending the process of gaseous exchange throughout the entire length of the vessel.

2. Under low rates of flow large areas of the capillary bed of the lungs may contain blood which is practically not in motion, the effective blood current being transmitted by other actively moving paths—paths determined, perhaps, by such extrinsic factors as respiratory movements or the force of gravity. An increase in the amount of blood delivered to such a lung may bring the sluggish areas into active circulation. This is the view advanced by Drinker, Churchill and Ferry (5). It has also been suggested by Toyama (6), although his experimental method is open to criticism because of the liability to embolism inherent in an injection method.

3. The increase in pressure in the pulmonary artery, which is the familiar result of an increased right ventricular output as well as the occlusion of one pulmonary artery, may bring about an anatomical widening, or actual opening, of reserve capillary paths. That the blood content of the vascular bed of the lungs actually does increase and decrease with varying rates of flow is shown by measurements of pulmonary blood volume under known rates of flow Drinker, Churchill and Ferry, (5). Further and important data on this point may be expected from a continuation of the direct observations of the capillary bed of the lung by Wearn, Barr and German (7). They have described the appearance and disappearance of individual capillaries around a single alveolus.

4. Capillaries may actively open through the agency of their inherent function of contractility. Evidence concerning the occurrence of such a phenomenon may be forthcoming from studies in direct microscopic observation. To prove that the capillaries of the lung open and close by virtue of their own contractile powers, observations must be carried on with a rigid control of the volume output of the right ventricle in order to exclude the action of the rise in pulmonary artery pressure which accompanies an increase in blood flow. Intra-alveolar air pressure changes which may readily result from variation in the diameter of the bronchi, must also be controlled, as this plays directly upon the thin walls of the surrounding capillaries. Drinker, Churchill and Ferry (5) measured the volume of

blood in the lungs under conditions including high carbon dioxide and low oxygen concentrations, and found no detectable change.

It was stated at the outset that the two responses of the lung to an increased functional demand—an alteration in breathing and an increase

TABLE 4

*Effect of exercise on oxygen consumption and pulmonary ventilation in human subjects. Calculated per square meter of body surface area. (Peabody and Sturgis, 1922)*

| NORMAL SUBJECTS | PERIOD I—REST | | | PERIOD II—EXERCISE | | |
|---|---|---|---|---|---|---|
| | Oxygen consumption | Minute volume | $\frac{O_2 \text{ consumption}}{\text{Minute volume}}$ | Oxygen consumption | Minute volume | $\frac{O_2 \text{ consumption}}{\text{Minute volume}}$ |
| | *cc.* | *cc.* | | *cc.* | *cc.* | |
| J. D. T. | 166 | 4,150 | 0.040 | 420 | 8,260 | 0.050 |
| F. F. S. | 125 | 3,060 | 0.040 | 508 | 8,250 | 0.061 |
| L. L. | 171 | 4,050 | 0.042 | 474 | 7,640 | 0.062 |
| H. B. | 157 | 4,010 | 0.039 | 376 | 7,840 | 0.047 |
| F. W. P. | 145 | 3,630 | 0.039 | 445 | 8,020 | 0.055 |
| F. R. S. | 167 | 3,940 | 0.042 | 468 | 9,350 | 0.050 |

TABLE 5

*Effect of increasing blood flow on oxygen consumption and pulmonary ventilation of one lung of normally breathing anesthetized cats*

| CATS | PERIOD I—CONTROL | | | PERIOD II—VOLUME FLOW OF BLOOD INCREASED BY OCCLUDING OPPOSITE PULMONARY ARTERY | | |
|---|---|---|---|---|---|---|
| | Oxygen consumption | Minute volume | $\frac{O_2 \text{ consumption}}{\text{Minute volume}}$ | Oxygen consumption | Minute volume | $\frac{O_2 \text{ consumption}}{\text{Minute volume}}$ |
| | *cc.* | *cc.* | | *cc.* | *cc.* | |
| Cat 1 | 6.0 | 290 | 0.021 | 11.0 | 346 | 0.032 |
| Cat 2 | 16.0 | 525 | 0.030 | 26.0 | 566 | 0.045 |
| Cat 3 | 12.5 | 359 | 0.034 | 27.0 | 591 | 0.045 |
| Cat 4 | 15.0 | 788 | 0.018 | 28.8 | 788 | 0.036 |
| Cat 5 | 12.5 | 706 | 0.017 | 27.5 | 738 | 0.037 |
| Cat 6 | 12.0 | 288 | 0.041 | 22.0 | 356 | 0.061 |

in the diffusing surface—were closely linked. In fact, in the range of normal physiological processes the two compensatory measures doubtlessly always act together, and it is only through artificially imposed experimental methods that they can be separated. It seems needless to point out that

an increase in the diffusing surface brought about solely by the dynamics of the pulmonary circuit, would also take place in the relatively poorly ventilated areas. Under such conditions, a return of a larger fraction of unarterialized blood to the left heart would be the result.

In conclusion, it is of interest to review what is known to happen in human subjects in response to the demand for increased oxygenation which accompanies exercise. Table 4 has been compiled from figures on oxygen consumption and pulmonary ventilation given by Peabody and Sturgis (8). In the first period the subjects were standing quietly at rest. The second period followed the first after about two minutes, and in this period the subjects walked sixty steps upstairs on a treadmill in one minute. The height of each step of the treadmill was 18 cm. The rate of blood flow was not recorded in these studies, but the work of Bock and his co-workers provides ample evidence for us to assume an increase of blood flow with such exercise.

By comparison with table 5 which is a condensed summary of the experimental observations recorded above, we may see that the same increase in the effectiveness of the pulmonary ventilation may be obtained in the experimental animal by the simple expedient of increasing the volume flow of blood through the lung.

It appears, therefore, that the lung, by virtue of the altered dynamics of the pulmonary circuit which attend an increased rate of blood flow, is able to increase the effectiveness of its diffusing surface. This mechanism in turn helps to satisfy the increased oxygen demand usually associated with physiological increases in blood flow.

## SUMMARY

A preparation is described in which the ventilation and oxygen absorption of each lung of a normally breathing cat may be recorded. Systemic blood pressure and pulmonary artery pressures are also taken simultaneously. By occluding one branch of the pulmonary artery, or one set of pulmonary veins the total output of the right ventricle may be diverted to the contralateral lung.

The usual response to the demand for increased function so produced is an increase in the respiratory minute volume. This response is slight, however, in comparison with the marked increase in oxygen absorption which takes place. In fact, the increase in oxygen absorption is shown to take place even when the respiratory minute volume remains the same or is actually decreased. It seems probable, therefore, that an increased blood flow in a lung, increases the functional diffusing surface. Mechanisms by which this may be accomplished are briefly outlined. Available figures concerning the oxygen consumption and pulmonary ventilation in human subjects during exercise are quoted and compared with the

results obtained by experimentally increasing the blood flow through one lung of a cat.

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# THE EFFECT OF VARIATION IN POSTURE ON THE OUTPUT OF THE HUMAN HEART

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Received for publication June 25, 1928

The purpose of the present investigation was the study of the effect of assuming various postures on the output of the human heart in normal individuals. This problem is of both practical and theoretical importance because of its bearing on the results obtained in determinations of the cardiac output as well as because of its bearing on the normal behavior of the human heart and the mechanism of its regulation. The effect of varying postures on the output of the heart has been the subject of experiment by almost every method heretofore devised for determining the cardiac output in man. Chief among these are to be mentioned the work of Lindhard (1913), Field and Bock (1925), Lawrence, Hurxthal and Bock (1927), Turner (1927), Henderson and Haggard (1925), Rosen and White (1926), and Davies and Gilchrist (1926–27). Despite this apparently adequate attack of the problem, scrutiny of the results obtained by these investigators shows many incongruities.

Lindhard (1913), utilizing the nitrous oxide method of Krogh and Lindhard (1912, 1913), found a definite sex-difference in the subjects whom he studied. The minute volumes were 5.0 per cent, 16.2 per cent and 30.3 per cent higher in the cases of the 3 female subjects in the recumbent than in the sitting posture. In the cases of the four male subjects studied, change in posture produced only slight variations in the cardiac output. Collett and Liljestrand (1924) also using the Krogh-Lindhard method record experiments on one female subject and found 4.9, 4.0 and 3.6 liters as the cardiac output for the erect, sitting, and recumbent postures, respectively, in agreement with the findings of Lindhard.

Field and Bock (1925), utilizing the method described by Field, Bock, Gildea and Lathrop (1924) in 13 determinations, found that the average cardiac output in the sitting position was 76 per cent and in the erect position only 50 per cent of that found when the subject was in the recumbent position. The output per beat was only 35 per cent as great in the standing as in the recumbent position. All but one of Field and Bock's subjects were males. Lawrence, Hurxthal and Bock (1927), in a later communication, extended the work of Field and Bock (1925) and confirmed the latter's

findings. Turner (1927), on the other hand, in a series of determinations on 25 female subjects and by the use of the same method as Field and Bock, failed to find as marked changes as are reported by the latter investigators. She found the average decrease in the sitting posture to be 11 per cent and in the standing 21 per cent as compared with the results in the recumbent posture. Henderson and Haggard (1925), in the original presentation of the ethyl iodide method, report a few results on the cardiac output in different postures showing a great increase in the recumbent over the erect posture. Rosen and White (1926) with the same method confirm the findings of Field and Bock. As pointed out by Rosen and White, "the circulation rate obtaining in the recumbent position may be as great as when the subject is taking light exercise." Davies and Gilchrist (1926–27), on the other hand, also using the ethyl iodide method, did not always find the marked differences accompanying change in posture which are reported by Henderson and Haggard and Rosen and White. They, therefore, refuse to express any definite conclusion as to the effect of posture on the circulation rate. Indeed, the average value of a series of ten observations on one of their subjects in the supine position and the average of 9 estimations in the sitting position agreed within the experimental error. Douglas and Haldane (1922), using the method of Christiansen, Douglas and Haldane (1914), found, in one subject, a cardiac output per minute of 7.5 and 6.8 liters in the recumbent position as compared to 8.0 and 8.4 liters, respectively, in the sitting posture.

This review of the past work on the effect of posture on the cardiac output suffices to show the diversity in the results obtained, not only by different methods but even by the same method when employed by different observers. The generally accepted view at present is that the cardiac output is higher in the recumbent than in the erect posture. However, the inconclusiveness of the available data, the doubtful accuracy of the methods employed, and the importance of the subject warranted a reinvestigation of the entire matter. The present paper presents this study and aims to demonstrate that the generally accepted views concerning the effect of posture on cardiac output are in error and that, in contrast to the conclusions of previous workers, the cardiac output of normal individuals, male or female, is independent of posture and is essentially the same in the recumbent, sitting and erect postures.

METHOD. The method employed in determining the output of the heart in this investigation was that previously described by Marshall and the author (Marshall and Grollman, 1928). Nitrous oxide was used in all of the experiments. The subjects rebreathed a mixture of nitrous oxide, oxygen and air, for 25 seconds. In the earlier experiments the samples for analysis were collected at 15 and 23 seconds after the beginning of the rebreathing but in the later experiments these collections were made at 18 and 23 seconds, for reasons to be discussed below.

The subjects were all healthy adults from twenty to thirty years of age. Many of the experiments were done on subjects in the basal condition. These subjects were studied on their arrival at the laboratory in the morning. In view of the possible effects of drinking liquids on the cardiac output (Jarisch and Liljestrand, 1927), they abstained from drinking water or other liquids before the experiment. The subjects rested for one-half hour before beginning the determinations. Some experiments were made on subjects not in the basal condition. These experiments were done late in the afternoon, four or five hours after a light luncheon and the subjects, who had in no case been doing any physical work for several hours, were allowed a half-hour of complete rest before the experiment.

The following procedure was carried out in a series of determinations. The subject lay prone on a comfortable cot for one-half hour in the experiments in which the first determination was made in the recumbent position. In order to avoid the possibility of this resting period in the recumbent position affecting the final comparative results, some of the experiments were carried out first with the subject in the sitting position. In these cases, the subject sat quietly in an ordinary, straight-backed chair with feet on the floor during the preliminary rest-period. At the conclusion of this period, the mouthpiece of a Krogh metabolism spirometer was put into the subject's mouth without permitting the subject to change his position. The mouthpiece and valve of the metabolism apparatus were suspended from the ceiling and adjusted to the required height. After the completion of the determination of the subject's oxygen consumption, five minutes were allowed to elapse in which the subject again equilibrated his system with the atmosphere. The cardiac output was then determined by the previously described method (Marshall and Grollman, 1928). The pulse was taken by radial palpation at various intervals during the experiment. After this determination in the recumbent or sitting posture, the experiment was repeated in another position. After maintaining the new position for eight minutes another determination of the oxygen consumption was made and after five more minutes, a second cardiac output was determined. The subject was then transferred to the third position and the process again repeated. In some experiments determinations were made in only two positions. The time necessary for a complete experiment in each position, was about twenty minutes, the complete duration of a series of determinations in all of the positions being about one hour exclusive of the time allowed for the preliminary period of rest. The order of the experiments was reversed, as described above, in order to avoid the possibility of a change in heart output with time but no such difference could be detected. When the subjects were in the erect position they stood *at ease* and in as comfortable an attitude as they could assume. In this way the fatigue and fainting, accompanying the erect posture when maintained *at attention*, were avoided.

RESULTS. The results of these determinations are given in tables 1 to 4. Tables 1 and 2 each give a series of determinations on the same individual under varying conditions. These experiments were carried out to determine the results obtained under other than the fasting and resting conditions which obtained in the case of the subjects of tables 3 and 4.

TABLE 1

*The effect of variation of posture under different conditions on the cardiac output, pulse and oxygen consumption, of a male subject, A. G.; age, 26; height, 164 centimeters; weight, 63 kilograms*

| TIME AND DATE OF EXPERIMENT | CONDITION OF SUBJECT | RECUMBENT | | | SITTING | | | STANDING | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume |
| *1928* | | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* |
| February 10, 5 p.m. | Fatigued and feeling badly after day of hard work | 76 | 259 | 3.2 | 78 | 330 | 3.4 | 88 | 353 | 3.4 |
| February 11, 6 p.m. | 5 hours after last meal | 68 | 260 | 4.0 | 76 | 260 | 3.8 | 88 | 278 | 3.8 |
| February 16, 9 a.m. | Basal | 68 | 211 | 4.5 | 73 | 248 | 4.5 | 88 | 330 | 3.7* |
| February 23, 8 p.m. | Light meal at 5 p.m. | | | | 88 | 287 | 3.4 | 100 | 315 | 3.3 |
| February 29, 7 p.m. | Heavy meal at 6 p.m. | 74 | 258 | 4.1 | | | | 90 | 320 | 4.0 |
| Average† | | 71 | 243 | 3.9 | 76 | 279 | 3.9 | 88 | 320 | 3.6 |
| Variation of average from recumbent value | | | | | +7% | +15% | 0.0% | +24% | +32% | −8% |

* Subject became giddy but did not faint.

† Includes only those values in which determinations in all three postures were made.

DISCUSSION OF RESULTS. An examination of the data given in tables 1, 2, 3 and 4 shows that posture has relatively little effect on the cardiac output. The average change in the cardiac output in changing from the recumbent to the sitting or erect posture is, in the case of the subjects of all 4 tables, 8 per cent or less. Considering the unavoidable error, inherent in the determinations of the cardiac output and the possibility of slight

changes in metabolism or cardiac output occurring during the course of the procedure, it must be concluded that within the experimental error (± 10 per cent), the variation of posture is without effect on the cardiac output. Of the subjects studied only one (L. G., table 3) shows a decrease in the cardiac output in the sitting posture that is more than 10 per cent less than the value obtained in the recumbent position. This subject was

TABLE 2

*The effect of variation of posture, under different conditions on the cardiac output, pulse and oxygen consumption of a female subject, A. L.; age, 28; height, 158 cm.; weight, 55 kilograms*

| TIME AND DATE OF EXPERIMENT | CONDITION OF SUBJECT | RECUMBENT | | | SITTING | | | STANDING | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume |
| *1928* | | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* |
| February 4, 5 p.m. | 4 hours after light luncheon | 58 | 193 | 3.5 | 60 | 229 | 3.7 | 64 | 305 | 3.9 |
| February 6, 6 p.m. | 5 hours after light luncheon | 58 | 208 | 3.1 | 60 | 245 | 3.2 | 64 | 248 | 3.2 |
| February 12, 5 p.m. | 4 hours after light luncheon | 54 | 245 | 3.8 | 60 | 246 | 4.0 | 70 | 280 | 3.4 |
| February 19, 1 p.m. | Basal | 59 | 199 | 2.4 | 64 | 218 | 2.6 | 78 | 225 | 2.5 |
| February 19, 9 p.m. | 1 hour after heavy meal | 69 | 275 | 3.8 | | | | 80 | 322 | 3.7 |
| Average*..... | | 57 | 211 | 3.20 | 61 | 235 | 3.38 | 69 | 265 | 3.25 |
| Variation from recumbent value..... | | | | | +7% | +11% | +6% | +21% | +26% | +2% |

* Excludes last experiment in which no determination was made in the sitting posture.

studied on three occasions in a period of 2 months and in every case the same results were obtained.

During the course of the early part of this investigation, it became apparent that the magnitude of the results obtained for the standing posture agreed in general with those previously reported by other workers. The results, however, differed from those hitherto reported in that the values

for the recumbent and sitting positions were low and equal to those obtained in the erect position, whereas previous workers had found successively higher results for the sitting and recumbent postures. In several experiments the preliminary rebreathing, required in carrying out the present method for determining the circulatory minute volume, was inadequate for obtaining homogeneous mixture. The results indicated much higher values for the recumbent than for the sitting or erect postures. This find-

TABLE 3

*The effect of variation of posture on the cardiac output, pulse and oxygen consumption of normal men*

| SUBJECT | CONDITION | HEIGHT | WEIGHT | RECUMBENT | | | SITTING | | | STANDING | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume |
| | | *cm.* | *kgm.* | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* |
| S. J. W. | Non-basal | 180 | 66 | 66 | 265 | 4.1 | 70 | 267 | 4.0 | 72 | 292 | 4.1 |
| L. M. | Non-basal | 168 | 57 | | | | 90 | 264 | 4.1 | 100 | 292 | 3.9 |
| L. M. | Non-basal | 168 | 57 | 86 | 195 | 4.3 | | | | 92 | 250 | 4.2 |
| I. J. W. | Non-basal | 176 | 72.5 | 58 | 258 | 6.1 | | | | 83 | 420 | 6.0 |
| C. L. G. | Basal | 179 | 78 | 51 | 265 | 4.7 | 53 | 270 | 4.8 | | | |
| L. G. | Basal | 180 | 72 | 64 | 236 | 4.3 | 70 | 265 | 3.6 | 80 | 267 | 3.5 |
| L. G. | Basal | 180 | 72 | 64 | 236 | 4.4 | 70 | 236 | 3.5 | 86 | 263 | 3.5 |
| A. F. D. | Non-basal | 179 | 81 | 56 | 251 | 3.4 | 62 | 265 | 3.4 | | | |
| G. C. | Non-basal | 180 | 63.5 | 89 | 257 | 6.4 | 90 | 257 | 5.9 | | | |
| S. W. B. | Basal | 170 | 77 | | | | 70 | 295 | 4.0 | 88 | 318 | 3.8 |
| S. W. B. | Basal | 170 | 77 | 68 | 260 | 4.3 | 76 | 260 | 4.1 | 92 | 265 | 4.2 |
| A. G. | Non-basal | 164 | 68 | 68 | 260 | 4.0 | 76 | 260 | 3.8 | 88 | 278 | 3.8 |
| Average*..... | | | | 67 | 255 | 4.2 | 73 | 263 | 3.9 | 83 | 276 | 3.9 |
| Variation from recumbent value..... | | | | | | | +9% | +3% | −7% | +24% | +8% | −7% |

* Excludes determinations in which experiments in all three postures were not made and the duplicate determination on A. L. G.

ing led to a closer investigation of the respiratory factors involved in postural changes. The results of this study showed that these postural effects on purely respiratory mechanisms are often very great and must be considered in those determinations of the cardiac output which are dependent upon respiratory maneuvers. Since every method heretofore used for determining the cardiac output in man involves such respiratory maneuvers it is necessary to consider the effect of posture on the respiratory mechanism

in determinations of the circulatory minute volume. Indeed, as shall be shown later, it is the neglect of these factors that has, in part at least, led to the erroneous conclusions regarding the effect of variation of posture on the cardiac output which the previous observers, mentioned in the introduction to this paper, have reported.

THE EFFECT OF POSTURE ON THE VITAL CAPACITY, COMPLEMENTAL, RESERVE AND RESIDUAL AIR. Hutchinson (1846) observed that the position of the body had an effect on the respiratory movements of the chest wall, and later (1849–52) records that the vital capacity is less in the recumbent than in the erect posture. Panum (1868) showed that the volume of the

TABLE 4

*The effect of variation of posture on the cardiac output, pulse and oxygen consumption of normal women*

| SUBJECT | CONDITION | HEIGHT | WEIGHT | RECUMBENT | | | SITTING | | | STANDING | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume | Pulse | Oxygen consumption | Circulatory minute volume |
| | | *cm.* | *kgm.* | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* | | *cc. per minute* | *liters* |
| M. C. | Basal | 140 | 63 | 64 | 198 | 3.2 | 64 | 209 | 3.0 | 68 | 214 | 3.2 |
| M. H. | Non-basal | 171 | 62.5 | 60 | 244 | 4.1 | 70 | 253 | 3.8 | 84 | 314 | 3.8 |
| W. H. | Non-basal | 171 | 54.5 | 78 | 219 | 4.3 | 81 | 236 | 4.2 | 88 | 274 | 3.6 |
| E. W. | Basal | 167 | 63.5 | 60 | 248 | 3.5 | 64 | 261 | 3.5 | 74 | 276 | 3.5 |
| J. P. | Basal | 155 | 49 | 64 | 158 | 3.2 | 68 | 173 | 3.3 | 71 | 207 | 3.3 |
| A. C. | Basal | 158 | 55 | 59 | 199 | 2.4 | 64 | 218 | 2.6 | 78 | 225 | 2.5 |
| Average | | | | 64 | 209 | 3.45 | 68 | 225 | 3.40 | 77 | 252 | 3.33 |
| Variation from recumbent value | | | | | | | +6% | +8% | −1% | +20% | +21% | −3% |

reserve air and the vital capacity were less in the sitting and recumbent than in the standing postures. Lovén (1872) could detect no difference between the standing and sitting postures but found usually a decrease in the recumbent position. The first complete study of the effect of posture on various respiratory functions was that of Bohr (1907). This investigator found that the vital- and the mid-capacities are decreased in the recumbent position; hence, the reserve air is lower in this position while the complemental air is higher than in the sitting or erect postures. The change in vital capacity was immediate and is attributed by Bohr to changes in the mechanical factors involved in respiration. The change in the mid-capacity, on the other hand, occurs gradually and is attributed to a reflex action

similar to that which occurs in the case of the pulse rate and metabolism. The residual air (equal to the mid-capacity minus the reserve air) can be calculated from Bohr's data and is found to be increased in the recumbent posture. Haldane, Meakins and Priestley (1918–19) have shown that the recumbent position produces a tendency to anoxemia and a slowing and deepening of the respiration. Christie and Beams (1922–23), in a study of 290 normal individuals, found that 80 per cent of their subjects had a lower vital capacity in the recumbent than in the sitting position. Recently Wilson (1927), oblivious of the work of Bohr (1907), has reinvestigated the effect of posture on the volume of the reserve air. Wilson shows that "the air content of the lungs may be a liter less in the recumbent than in the erect posture and that, with every change in the position of the body, the base-line of normal respiration shifts to a different level." The volumes of the reserve air of four individuals in the erect posture were 1600, 1700, 2400 and 1460 cc. while in the recumbent posture these volumes were reduced to 650, 700, 800 and 590 cc., respectively. In a man of average vital capacity the reserve air may be reduced from 1550 cc. in the erect position to as little as 250 cc. in the recumbent position. Skiographic studies of the variation in the height of the diaphragm in the thorax with different postures give an explanation of these effects on the respiratory mechanism.

The importance of these findings to determinations of the cardiac output by methods in which alveolar samples or other respiratory maneuvers are required—requirements, by the way, of all heretofore described methods—will be apparent. Obviously with the reserve air reduced to the minimum, as it is in the recumbent position, the taking of alveolar samples in this posture, by the usual expiratory methods, becomes impossible. Determinations of the cardiac output involving such methods will be in error. Moreover, an evaluation of the magnitude of these purely mechanical factors is necessary before one can interpret the significance of the observed diminution in the $CO_2$ content of the alveolar and mixed venous air samples which have been reported (Higgins, 1914; Lawrence, Hurxthal and Bock, 1927; Turner, 1927) as occurring as one changes from the recumbent to the sitting and standing postures.

Effect of posture on mixing in the lungs. In the method for the determination of the heart output, as used in this investigation, the above described factors are secondary and enter into the consideration only in so far as they affect the ability of the subject to attain homogeneous mixture in the *lung-bag* system. The attainment of this mixture is essential and in cases where it is not attained the results are too high. In view of the marked effects which change of posture induces in the respiratory system, it cannot be assumed that mixing will be attained in the same period of time with the subject in the recumbent as in the sitting or standing postures. In a previous paper this problem was studied for the sit-

ting posture only (Grollman and Marshall, 1928). Experiments were, therefore, carried out to determine the relative times necessary for attaining homogeneous mixture in the *lung-bag* system in varying postures. Because of the importance of the problem to rebreathing experiments in general, the results will be reported here. The experimental method employed was similar to that used in the previous study, successive determinations being made with the subject in varying postures. The results

TABLE 5

| SUBJECT | POSTURE | VOLUME IN BAG | TIME OF REBREATHING | NUMBER OF REBREATHINGS | DIFFERENCE IN CONCENTRATION BETWEEN "ALVEOLAR" AND BAG SAMPLES |
|---|---|---|---|---|---|
| | | *liters* | *seconds* | | *per cent of hydrogen* |
| A. G., ♂ | Recumbent | 2.4 | 12 | 4 | +0.007 |
| | Sitting | 2.4 | 12 | 4 | +0.045 |
| | Recumbent | 2.4 | 15 | 3 | −0.012 |
| | Sitting | 2.4 | 15 | 3 | −0.009 |
| | Recumbent | 2.0 | 15 | 5 | +0.009 |
| | Sitting | 2.0 | 15 | 5 | +0.030 |
| L. M., ♂ | Standing | 2.0 | 14 | 7 | +0.015 |
| | Standing | 2.0 | 15 | 6 | +0.022 |
| | Recumbent | 2.0 | 15 | 7 | −0.020 |
| | Recumbent | 2.0 | 18 | 9 | +0.024 |
| S. J. W., ♂ | Standing | 2.0 | 15 | 9 | +0.010 |
| | Recumbent | 2.0 | 15 | 9 | −0.004 |
| | Recumbent | 2.0 | 18 | 9 | +0.012 |
| A. L. C., ♀ | Recumbent | 2.0 | 13 | 6 | −0.021 |
| | Sitting | 2.0 | 13 | 6 | +0.002 |
| | Standing | 2.0 | 13 | 6 | +0.017 |
| | Standing | 2.4 | 15 | 6 | +0.006 |
| | Recumbent | 2.4 | 15 | 6 | −0.008 |
| | Recumbent | 2.0 | 15 | 6 | +0.032 |
| | Recumbent | 1.8 | 15 | 6 | +0.010 |

are given in table 5. A 4 per cent mixture of hydrogen gas in air was used in all of the experiments. The last column of table 5 shows the difference in concentration of the hydrogen in an alveolar sample obtained at the end of a forced expiration and that present in the bag. When this difference is positive, mixing may be considered as having been obtained; negative values indicate inadequate mixture.

It will be seen from table 5 that the ability to attain homogeneous mix-

ture in the *lung-bag* system is less in the sitting than in the standing position and least in the recumbent. This difference is pronounced. Although most subjects are capable of attaining the required mixture under given conditions in 15 seconds when in the sitting position (Grollman and Marshall, 1928), this is not true when the subject assumes the recumbent posture. In the latter position a minimum rebreathing period of 18 seconds is necessary for ensuring adequate mixture. The reason for this fact becomes quite obvious when we consider the changes produced in the mechanical factors involved in respiration as discussed in the preceding section.

THE EFFECT OF INADEQUATE MIXTURE ON THE RESULTS OF BLOOD FLOW DETERMINATION. The above consideration of changes in respiratory conditions with posture resulted from determinations on two subjects, L. S. W. and L. M., in whom the circulatory minute volume in the recumbent position was higher than that in the sitting or erect postures, as had been found by previous investigators. The fact that with incomplete mixture one usually obtains results which are too high led to the investigation which has been reported above. In the subjects whose cardiac output in the recumbent position was greater than that in the sitting or standing positions, it was found that mixture was in every case incomplete in the recumbent position after 15 seconds' rebreathing, as is shown in table 5 (subjects L. M. and L. S. W.). Adequate mixture is attained in these subjects in the recumbent position only after rebreathing for 18 seconds. On repeating the determinations and collecting the samples at 18 and 23 seconds after the beginning of the rebreathing period instead of at 15 and 23 seconds, as had been done before, the results recorded in table 3 were obtained. The fact that the results obtained when mixture is incomplete agree so closely with those obtained by previous workers who have studied the effect of variation of posture on the circulatory minute volume, makes it perhaps worth while to record several examples (table 6).

TABLE 6

*The effect of inadequate mixture on the apparent circulatory minute volume*

| SUBJECT | TIME OF COLLECTION OF SAMPLES | CIRCULATORY MINUTE VOLUME | |
|---|---|---|---|
| | | Recumbent | Erect |
| | *seconds after beginning of rebreathing* | *liters* | *liters* |
| L. M. | 15 and 25 | 4.8 | 3.9 |
| | 18 and 25 | 4.3 | 4.2 |
| L. J. W. | 15 and 25 | 6.5 | 4.6 |
| | 18 and 25 | 4.1 | 4.1 |
| A. G. | 10 and 23 | 5.0 | |
| | 15 and 23 | 3.5 | |
| | 18 and 23 | 3.6 | |

The experiments on subjects L. M. and L. S. W. were repeated on different days. When the first sample was collected after 15 seconds' rebreathing, before adequate mixture was obtained, 20 and 35 per cent higher values were obtained in the recumbent than in the erect position. When the first sample was collected at 18 seconds, by which time adequate mixture was obtained, the values in the recumbent and in the erect postures were the same. The three experiments on A. G. were performed at the same sitting. This subject can adequately mix (as will be seen from table 5) in 15 seconds; but cannot in 10 seconds. The value obtained when mixing is inadequate is almost 50 per cent higher than the true value.

In applications of this method of determining the circulatory minute volume in the recumbent posture, it is necessary to prolong the preliminary rebreathing period to 18 seconds. That prolongation of the preliminary period to 18 seconds does not introduce an appreciable error, due to the return of blood to the lungs, has been previously considered (Marshall and Grollman, 1928) and is evidenced by the fact that in this work where in the case of some subjects who could attain adequate mixture in 12 seconds, identical results were obtained when the collections were made at 12 and 18, 15 and 23 or 18 and 25 seconds, respectively.

CRITICISM OF PREVIOUS WORK. The effect of posture on the mechanical factors of respiration and the erroneous results obtained when due consideration of these factors are not considered, have been discussed in some detail because it seems probable that the errors involved in neglecting these facts explain, in great part, if not entirely, the results of previous investigators. Since it is at present most generally assumed, on the basis of this previous work, that variation in posture has a marked influence on the circulatory minute volume, it is necessary to consider in what manner these results are most probably in error.

Lindhard (1913) found, as a result of his studies, that the cardiac output was increased only in the three female subjects whom he studied. In another paper (Marshall and Grollman, 1928) mention has been made of the fact that the values obtained by the Krogh-Lindhard procedure as used by Lindhard show a greater variation, due probably to slight inadequacy of mixing and the difficulty of obtaining true alveolar samples, than do the results obtained by the procedure used in the present investigation. If this be true, it would be expected that this inaccuracy would be increased in the recumbent position and particularly in the case of women, especially if obese, since the effect of variation of posture on the respiratory functions in these cases is very marked. The problem of mixture in the *Krogh-Lindhard* procedure has also been studied by Weiss (1928). Liljestrand and Stenström (1925) in 42 determinations on seven normal women in the recumbent position found an average value of 3.8 liters. An average value of 3.5 liters was found in the present investigation, which represents a decrease of less than

10 per cent over the value of Liljestrand and Stenström. The results of these two series of subjects are, however, not truly comparable because of possible differences in age and general physical conditions of the two series as well as the relatively small number of subjects considered. Nevertheless, the values found by Liljestrand and Stenström indicate only a slight increase (if any) in the recumbent posture as compared to the sitting.

The largest series of subjects heretofore studied for the effect of variation in posture on the cardiac output are reported by Field and Bock (1925). These authors applied the Fick principle in their method by determining the arterio-venous carbon dioxide difference and the rate of elimination of carbon dioxide from the body. Although theoretically plausible, the results from a practical standpoint are open to serious objection. Several determinations are made and the average of these taken as the true arterio-venous carbon dioxide difference. In practice it is found that although on some individuals, the results which are averaged to obtain the final value may be consistent, in many cases individual determinations show a rather wide variation. It seems unjustified, therefore, to assume that the accuracy of the final value is much greater than the possible variations of the 4 or 5 individual determinations which are averaged. These errors are no doubt caused by respiratory difficulties, for example, the inability to obtain a true alveolar sample or a sample representative of the true venous blood. Moreover, from the considerations already discussed concerning the effect of posture on the respiratory function, these errors would be greatly exaggerated in the recumbent position. In fact, as has been pointed out before, it is probable that *in certain individuals a true alveolar sample is unobtainable by the usual procedures when the subject is in the recumbent posture.* An equilibration of the venous blood with a gaseous mixture would be equally difficult to attain in this position. Hence it would be expected that the most accurate results would be obtained in the erect posture, in which the respiratory conditions are optimal, as regards the ability of obtaining the necessary samples of pulmonary gases in equilibrium with the blood.

In accord with this conception, it is found that the values obtained by Field and Bock (1925) for the erect posture agree remarkably well with those obtained in the present investigation and with the series, determined in the sitting position, reported elsewhere (Marshall and Grollman, 1925). This leads one to the conclusion that the method of these workers may be accurate when done in the erect posture since in this position the respiratory conditions are such as to give the optimum conditions for carrying out their procedure. In the recumbent or sitting postures, due to the factors already outlined, fundamental errors are involved which invalidate the results. This, if it be true, is of some importance, for it means that this method, which is comparatively simple in its procedure,

would be of practical value wherever determinations in the erect posture are feasible.

Previous determinations of the effect of variation of posture on the cardiac output by means of the ethyl iodide procedure of Henderson and Haggard (1925) have been mentioned in the introduction to this paper. It seems unnecessary in view of the fundamental objections (Starr and Gamble, 1926–27; Moore, Hamilton and Kinsman, 1926; Wright and Kremer, 1927; Henderson and Haggard, 1927; Barcroft, 1927) which have been raised against the accuracy of this method to consider in detail the absolute values obtained (Cullis, Rendel and Dahl, 1926; Ringer, 1927; Mobitz, 1927), but the increase in cardiac output in the recumbent over the erect posture, as found by this method, requires brief comment. It seems most probable that this observed effect of posture is to be attributed to the difference between the average tension of ethyl iodide to which the blood is exposed and the tension obtained in the Henderson automatic sampler, which Barcroft (1927) has shown may give results which are 25 per cent too high. This error will be still further increased in the recumbent position for the reasons discussed in a previous section. We may account, therefore, for the difference in the sitting, erect, and recumbent postures, observed by Henderson and Haggard (1925) and Rosen and White (1926) by the increase in the magnitude of this error in changing from the standing to the sitting and from the latter to the recumbent posture. Repetition of the work, with adequate control (if possible) of all the factors advanced against the method, is necessary before these findings can be considered as valid.

PHYSIOLOGICAL CONSIDERATIONS. In conclusion we may briefly consider the physiological implications involved in the assumption of a constancy in the circulatory minute volume with varying postures as found in this investigation and the relation of this constancy to other factors influenced by postural changes. In changing from the horizontal to the vertical position, the most fundamental change in the organism which has occurred is the sudden shifting of a column of blood, the length of the body, from a horizontal to a vertical position where it exerts an hydrostatic pressure which it did not exert previously. Change in posture also produces marked changes in the respiratory mechanism and these must not be overlooked in considering the final effect on the circulatory minute volume. The constancy of the output of the heart and the ability of the human organism to compensate for the effect of the force of gravity is consistent with the investigations of Leonard Hill, Barnard and Soltaui (1895, 1897) who studied the effect of posture on the arterial and venous pressure in the dog, cat, monkey and man. They found a greater degree of development of the compensatory mechanism, with postural changes, in monkeys than in dogs or cats. We would expect, therefore, to find this mechanism most highly developed in man. There are three chief factors which we may

consider as bringing about this compensatory mechanism for overcoming the force of gravity and maintaining a constant circulatory rate independent of the position of the body. Of prime importance, as pointed out by Hill and Barnard (1897), is the maintenance of tone by the splanchnic area which prevents the accumulation of blood in the regions where it would tend to accumulate as a result of gravity. The second factor is the change which occurs in the heart demonstrating itself in a change in pulse rate and blood pressure. Finally, the effect of respiratory changes, which help to raise the column of venous blood against the effect of gravity, must be considered. In this last category are to be included the changes in the rate and depth of respiration, the position of the diaphragm, and the other mechanical factors accompanying postural variation.

Examination of tables 1 to 4 shows that the differences in the metabolic rate in the recumbent, sitting and erect postures vary with different individuals. The average increase was 8 per cent for the ten men studied (table 3) and 21 per cent for the six women (table 4). The constancy of the cardiac output indicates that this change in the metabolic rate is not accompanied, however, by a corresponding change in the cardiac output.

It is of interest to consider the change of the arterio-venous oxygen differences with varying posture. These have not been given in the tables but are readily calculated by dividing the oxygen consumption by the circulatory minute volume. In fact, it is this oxygen difference which is actually determined in the experiment, the circulatory minute volume being in turn calculated from this value and the oxygen consumption. The arterio-venous difference was found to vary with postural change in such a way as to maintain a constant circulatory minute volume. This is contrary to what might be expected from superficial considerations. The oxygen tensions in the tissues are dependent on the circulatory minute volume and the arterio-venous oxygen difference; hence, we might expect to find the latter unchanged in order for the organism to maintain constant the internal environment. There is no evidence, however, for the existence of a constant arterio-venous oxygen difference and it would seem that this may vary within moderate degree without an increase in the circulatory minute volume.

The arterio-venous oxygen differences in the sitting position as found in this investigation are between 40 and 60 cc. per 100 cc. of blood, with an average closer to the higher value. This high arterio-venous difference corresponds to the results of all investigations which give blood flows between 3 and 5 liters per minute in the sitting position (Lindhard, 1915; Burwell and Robinson, 1924; Marshall and Grollman, 1928; *et al.*). Much lower arterio-venous differences correspond to the higher circulatory minute volumes found by the various forms of the Fick method (Douglas and Haldane, 1922; Field, Bock, Gildea and Lathrop, 1924; Field and Bock,

1925; Turner, 1927; *et al.*) and by the ethyl iodide method (Henderson and Haggard, 1925; Davies and Gilchrist, 1926–7; *et al.*). Assuming the accuracy of the lower values for the circulatory minute volume, any great decrease in the cardiac output accompanying the standing posture would lead to an increase in the arterio-venous oxygen difference to values higher than 100 cc., which seems improbable. Moreover, the extremely low arterio-venous oxygen differences which the latter group of investigators have found for the recumbent posture would represent an unnecessarily great and wasteful expenditure of energy on the part of the organism.

The commonly observed fainting or giddiness occurring in subjects in the erect posture has been explained as indicative of the decreased blood flow which was supposed to accompany the erect posture. Although giddiness or fainting must no doubt result from a marked decrease in the circulatory minute volume, it can not be taken as indicating a normally lower blood flow in the erect over the recumbent position. It must be remembered that in normal individuals (lack of tone of the splanchnic area accounting for the phenomena in the debilitated) circulatory embarrassment does not occur unless the subject stands "at attention" or assumes a rigid erect posture. When standing relaxed, the imperceptible but constant muscular movements suffice to propel the venous blood past the valves of the veins and thus maintain the circulation. With perfect rigidity, this venous return is prevented and the resulting stasis probably results in a decrease in cardiac output and the consequent fainting or giddiness. The phenomena may also be a result of a lack of tone in the splanchnic area but this explanation seems improbable in the case of normal individuals in whom rigidity or standing *at attention* seems to be necessary for the manifestations of fainting or giddiness.

Despite the great amount of work which has been done on the effect of posture on the blood pressure (for the literature of which consult Schneider and Truesdell, 1922), no definite generalizations are possible. It seems most probable, as was pointed out by Erlanger and Hooker (1904), that the systolic and diastolic pressures may either rise or fall on change of posture, depending upon the state of the subject. As a rule, however, the pulse pressure is diminished in the recumbent as compared to the erect posture. Sewall (1919) concluded from his observations that the diastolic pressure is higher and the pulse pressure lower, in the standing than in the recumbent position. Erlanger and Hooker (1904) suggest the product of the pulse pressure and the pulse rate as a function of the output of the heart and show the constancy of this product in different postures. The constancy of the cardiac output with variation in posture, as found in this investigation, would be in accord with this conception. Schneider and Truesdell (1922) in a statistical study of 2000 cases found as average means of the product of the pulse pressure by the pulse rate, 3461 and 3735 for the reclining and

standing positions, respectively. This, according to Erlanger and Hooker's interpretation, would indicate a slight rise in the cardiac output in the standing over the recumbent posture,—a view consistent with the present findings.

It is obvious that with a varying pulse rate as occurs with change in posture and a constant heart output as found in this investigation, there must be a decrease in stroke volume even under so physiologically a slight change as variation in posture.

In view of the findings of the present investigation, the theoretical considerations of Richards (1927), need not be considered. The recent experiments of Thompson, Dailey, and Alper (1928) on the effect of variation in posture upon the composition, volume, and velocity of the blood-flow, can be explained as due solely to changes occurring in the peripheral circulation without invoking an assumed change in blood-flow, as is done by these authors.

SUMMARY

The circulatory minute volumes of a series of normal men and women were determined in the recumbent, sitting and erect postures. Contrary to the findings of previous investigators, the results show that the circulatory minute volume is maintained constant despite variations in posture. The effect of posture on the respiratory mechanism is pointed out, and it is shown that homogeneous mixture in a *lung-bag* system is less easily obtained in the recumbent than in the standing posture. Certain physiological implications involved in the conception of a constancy in the circulatory minute volume with varying posture are discussed.

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# THE ELASTICITY AND DUCTILITY OF SKELETAL MUSCLE

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Received for publication June 25, 1928

Many years ago Blix (1) showed that if a muscle is rapidly stretched and then instantaneously released the elasticity, i.e., the ability to recover from distortion, is almost perfect. If, however, the load is not promptly removed there occurs a slow after-elongation which continues with decreasing rate for many hours. When a muscle which has been stretched for several minutes is unloaded, there occurs an instantaneous return to a length somewhat greater than the initial length and then a slow after-shortening which also persists at a decreasing rate for hours. Blix obtained similar after-lengthening and after-shortening from a model consisting of a spiral spring surrounded by a viscous substance contained in a rubber tube. He interpreted his results as indicating that a muscle is a viscous elastic body. A similar conclusion was reached by Gasser and Hill (2) on the basis of experiments of a different kind.

The after-lengthening does not restore the muscle to its original length within any reasonable time. Langelaan (3) has shown that when an atonic muscle is stretched it suffers a certain amount of permanent elongation. He defines elasticity as momentary yielding to a sudden stress; it is a reversible phenomenon, because the strain disappears entirely with the removal of the stress. On the other hand, what he calls plasticity is a slow yielding to a continuous stress and is irreversible. The property of retaining a permanent deformation caused by an external force after that force has ceased to act is the chief criterion of plastic bodies. In this sense an atonic muscle is plastic, since it undergoes a permanent elongation when it is made to support a moderate load for an appreciable length of time. This plasticity of the atonic muscle is not necessarily related in any way to the plasticity of the muscles in decerebrate animals as described by Sherrington. Only confusion can result from using the same word for both. For this reason we shall avoid the use of the term in Langelaan's sense and shall substitute the word ductility, meaning thereby the tendency to undergo permanent elongation when subjected to prolonged longitudinal stress.

METHOD. In these experiments we used the gastrocnemius muscle of white rats weighing about 150 grams. The muscle was isolated from the

central nervous system by section of the sciatic nerve. The living muscle was studied with its circulation intact. Urethane anesthesia was employed. The dose varied with the weight, 1 cc. of a 20 per cent solution of urethane being sufficient to produce complete anesthesia in a 150 gram rat.

The gastrocnemius was completely uncovered and dissected free up to its two heads of origin from the femur. Care was taken to leave intact the blood supply which enters the upper end of the muscle from the popliteal space. The achilles tendon was left attached to the calcaneus. A no. 30 soft iron wire was fastened tightly around this bone and formed into a loop to which the copper wire could be attached. The calcaneus was separated from the leg and foot and raised along with the gastrocnemius. The soleus was divided and the gastrocnemius freed up to its heads of origin. The sciatic nerve was cut. A special tightly fitting clamp was secured to the upper end of the femur in such a way as not to interfere with the femoral vessels.

The animal was secured by this clamp and some other supports to a heavy vertical steel rod set in one end of a horizontal steel bar. From the other end of this bar arose another heavy rod to which a strong muscle lever was attached. The strength of the apparatus was so great that it did not spring at all with the weights used in stretching the muscle. Since the writing lever had a ratio of 1:10 every centimeter of movement recorded on the kymograph corresponded to a change of 1 mm. in the length of the muscle.

The entire animal with the exception of the head and neck was immersed in a bath of Ringer's solution contained in a large rectangular dish of pyrex glass. The temperature of the fluid was kept at 38°C. with a maximum variation of 1° either way. The solution contained NaCl 0.9 per cent, KCl 0.037 per cent, $CaCl_2$ 0.01 per cent, and $NaHCO_3$ 0.01 per cent.

A no. 34 copper wire connected the calcaneus with the muscle lever; and the apparatus was so arranged that an intermediate pulley was not required. After the connections had been made, the achilles tendon was grasped with artery forceps and traction of about 300 grams was exerted through the tendon, calcaneus, and wire on the muscle lever. This served to tighten the points of attachment and to straighten the wire. In such work as this it is necessary to avoid the use of a thread and pulley. Even the best linen thread suffers considerable deformation when stretched. This led us astray in the earlier stages of our work (4). Fine copper wire offers sufficient resistance to bending to make it impracticable to pass it over a pulley when so much depends upon the position to which the unweighted lever returns after the load is removed.

The unweighted lever exerted a pull of 10 grams upon the muscle and the scale pan weighed 10 grams. The tracings were made on a slowly moving

kymograph (fig. 1). The position of the unweighted lever was first recorded, then the scale pan was gently lowered by hand until it hung supported from the lever. After one minute it was raised and placed on a support. One minute later the scale pan loaded with a 20 gram weight was again allowed to hang from the lever for 1 minute and then raised. This was repeated seven times with 1 minute intervals of stretching and recovery, the loads increasing by 20 gram intervals up to 140 grams. The last load including the scale pan was therefore 150 grams or, including the weight of the lever, 160 grams.

THE EXTENSIBILITY OF ATONIC MUSCLE. When the lever was loaded the muscle stretched at first rapidly and then more and more slowly. The instantaneous elastic stretch is responsible for the greater part of each down-stroke of the lever recorded in the tracing (fig. 1). The slow after elongation is responsible for the rounded angles and the downward slant of the lower connecting lines. The first part of the after elongation is quite

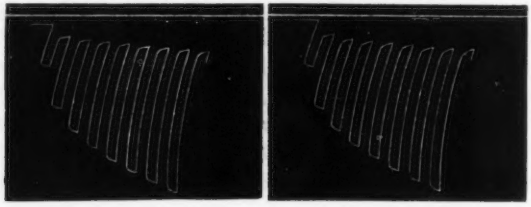

**Fig. 1. Tracings obtained from the gastrocnemius muscle of the white rat by alternately loading and unloading with weights increasing by 20 gram intervals and with minute periods of stretching and rest.**

rapid and it is impossible to say where this begins and where the primary elastic stretch ends. A more rapidly moving drum would show the after elongation more clearly but even in the tracings recorded by Blix (1) and Langelaan (3) it is not possible to make a sharp separation between the primary and secondary elastic phenomena.

When the load was removed the muscle shortened at first rapidly and then more and more slowly. This slow after elongation is responsible for the rounded angles and slight upward slant of the upper connecting lines. The first and more rapid part of the after shortening cannot be sharply distinguished from the primary elastic shortening. At the end of a minute, after removal of the load, the muscle had not returned to the height which it had before the loading. This is due to a more or less permanent elongation of the muscle which Langelaan called permanent deformation. Such tracings furnish evidence that a muscle is viscous as well as elastic, and, since it suffers a permanent elongation, it is plastic in the ordinary sense of the word; or, as we would prefer to say, it is ductile.

The permanent elongation caused by the first loading with the empty scale pan was considerable and highly variable in normal atonic muscle, and bore little relation to the amount of the deformation which developed later under the stress of heavier weights. The explanation of this is not clear; but it was not an instrumental error because it was not seen when muscles in rigor mortis or tetanus contracture were stretched. If one ignores this first variable stretch, the amount of deformation suffered by normal muscle, when stretched in the manner described by the addition of weights increasing by 20 gram intervals up to 140 grams, is slight but reasonably regular and constant (table 1).

In both tables the deformation recorded is the elongation that persisted one minute after the final lifting of the scale pan with its load of 140 grams, minus the deformation caused by the initial loading with the empty scale

TABLE 1

*Extensibility of the rat's gastrocnemius with circulation intact and sciatic nerve cut*

| NUMBER | WEIGHT | LENGTH | STRETCH | DEFORMATION |
|---|---|---|---|---|
| | *grams* | *mm.* | *mm.* | *mm.* |
| 17 | 1.47 | 30.8 | 3.92 | 0.50 |
| 18 | 0.99 | 31.0 | 4.05 | 0.47 |
| 19 | 1.08 | 29.0 | 3.79 | 0.54 |
| 20 | 0.78 | 25.5 | 3.84 | 0.47 |
| 21 | 0.79 | 27.1 | 3.40 | 0.44 |
| 22 | 0.95 | 26.9 | 3.85 | 0.37 |
| 23 | 0.87 | 28.0 | 3.89 | 0.40 |
| 24 | 0.99 | 28.0 | 3.86 | 0.42 |
| 25 | 1.08 | 26.9 | 4.05 | 0.48 |
| 26 | 0.99 | 27.0 | 3.59 | 0.40 |
| Average..... | 1.00 | 28.0 | 3.82 | 0.449 |

pan. The stretch recorded in the tables was that caused by the last loading (150 grams including the scale pan) and the amount was determined by measuring the last complete downstroke of the lever.

Since the resistance which an elastic body offers to a longitudinal stress depends in part upon its length and cross section, it was important to have accurate information about the size of the muscles used in the experiments. After the tracings were made the muscles were fixed under a tension of 25 grams in 10 per cent formalin for 24 hours or longer. Then the muscles were measured and weighed.

It will be seen from table 1 that there is no very obvious relation between the size of the muscle and the stretch or deformation. This is in part to be explained by the fact that an increased length which would favor the stretching is counterbalanced by increased weight and strength.

The normal atonic gastrocnemius of the white rat with intact circulation is capable of a very considerable elastic stretch, amounting on the average to 3.82 mm. in a muscle 28 mm. long when stretched with a weight of 150 grams. The deformation caused by the 7 successive loadings with weights increasing by 20 gram intervals was very slight, amounting on the average to 0.45 mm.

It will be evident that we have here a simple method by which we can obtain information concerning changes in the elasticity and ductility of muscles under various conditions. It is true that the tracings do not enable us to separate the primary elastic phenomena from the slow after-lengthening and after-shortening. And since the after-shortening continues at a

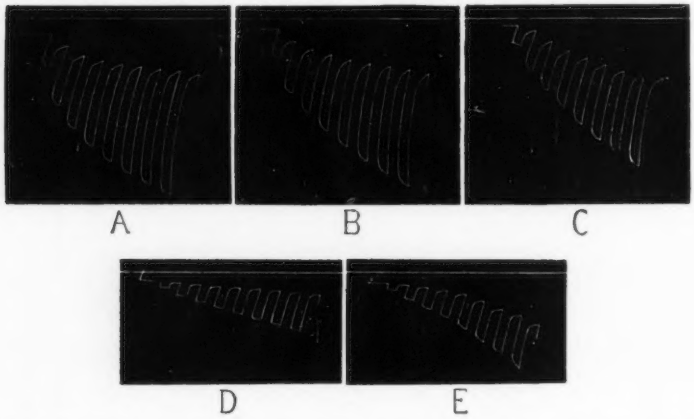


Fig. 2. Tracings obtained from the gastrocnemius muscle of the white rat at various stages of rigor mortis: A, from muscle M5R ¾ hour after death; B, from muscle M6R 1¼ hour after death; C, from muscle M4R 1½ hour after death; D, from muscle M2L 3½ hours after death; and E, from muscle M3R 4½ hours after death.

steadily decreasing rate almost indefinitely it is not possible to obtain exact figures for the permanent elongation and ductility of muscle. Nevertheless it is possible to show by means of such tracings that the elasticity and ductility of muscle are subject to marked changes under certain conditions such as rigor mortis and tetanus contracture.

Rigor mortis. Shortly after death marked changes occur in the elasticity and ductility of muscle. In Luciani's *Text Book of Physiology* we find the statement, "After death, when rigor mortis begins, muscle is highly elastic, that is, but little extensible, and its elasticity simultaneously diminishes, for when the traction is removed it no longer returns to its initial length." Bethe (6) also noted that one could easily cause a perma-

nent elongation of muscle in rigor mortis or heat rigor. Nakamura (7) using Bethe's elastometer found that the resistance against stretching decreased slightly just before the beginning of rigor mortis. As the rigor began the resistance increased and reached its maximum at the time of maximum shortening, after which it gradually decreased again.

The tracings reproduced in figure 2 show very well the relative parts played by primary elastic stretch, after-lengthening and permanent deformation in the extensibility of muscle in the various stages of rigor mortis. A muscle which was tested three-quarters of an hour after death (fig. 2 A) showed only a slight decrease in the primary elastic stretch but the after-lengthening and after-shortening were increased and prolonged, as is evidenced by the greater rounding of the angles and more marked slant of the short connecting lines in this tracing than in that of living muscle (fig. 1). There was also a slight increase in the permanent elongation. Similar results were obtained from a muscle tested 1¼ hour after death (fig. 2 B) but the after-lengthening, after-shortening and permanent elongation were more evident. At the time when this second tracing was being taken a slight stiffness could be noticed in the other limbs of the rat indicating that the onset of rigor mortis was imminent. Another muscle, tested one and a half hour after death, gave the tracing recorded at C, which shows a very evident decrease in the primary elastic stretch and increase in the permanent elongation. When the rigor was at its height three and one-half hours after death a tracing of quite a different character was obtained. In this muscle the primary elastic stretch was greatly decreased. After-lengthening and after-shortening were reduced to a minimum so that the short connecting lines in the tracing were almost horizontal and made sharp angles with the vertical lines (fig. 2 D). This muscle suffered less permanent elongation than is characteristic for living muscle when stretched in the same manner. The elasticity of a muscle at the height of rigor mortis is increased in the sense that it offers greater resistance than fresh muscle to a given longitudinal stress; and the elasticity is more nearly perfect in that the muscle suffers less permanent deformation than would a fresh muscle under the same conditions. But this state of affairs does not last long. An hour later the other gastrocnemius of the same rat (4½ hours post mortem) showed a marked increase in the permanent deformation although the elastic stretch remained small (fig. 2 E).

As will be seen from table 2, most of the rats used in the study of rigor mortis were slightly smaller than those used for the study of living muscle, and the gastrocnemius weighed correspondingly less. A detailed comparison of tables 1 and 2 will however show that this difference in weight was not responsible for the difference in results of the two series of experiments. In muscle M5R just before the onset of rigor mortis the stretch at the final loading amounted to 3.3 mm. Living muscle under the same con-

ditions would stretch 3.8 mm. The stretch decreased more and more until rigor was fully developed when it amounted to only 1.05 mm., or less than one-third normal. As the muscle begins to pass out of rigor four or five hours after death the stretch increases somewhat; but as can be seen from figure 2 E this is largely, if not entirely, due to an increase in the after elongation, which has its counterpart in an increased permanent deformation. This deformation amounted to 0.81 mm. in a muscle just before the onset of rigor mortis, reached 1.33 mm. in a muscle tested one and one-half hours after death, rapidly decreased to 0.11 mm. two and one-half hours after death and then increased again to 4.23 mm. in a muscle tested five and one-quarter hours post mortem.

TABLE 2

*Extensibility of the rat's gastrocnemius in rigor mortis*

| MUSCLE | HOURS POST MORTEM | WEIGHT | LENGTH | STRETCH | DEFORMATION |
|---|---|---|---|---|---|
| | | *grams* | *mm.* | *mm.* | *mm.* |
| M5R | ¾ | 0.89 | 25.1 | 3.30 | 0.81 |
| M6R | 1¼ | 0.72 | 22.1 | 3.10 | 0.92 |
| M4R | 1½ | 0.76 | 25.0 | 2.45 | 1.33 |
| M7R | 1¾ | 0.89 | 24.0 | 1.46 | 0.48 |
| M4L | 2¼ | 0.73 | 25.4 | 1.28 | 0.73 |
| M1R | 2½ | 1.08 | 25.4 | 1.06 | 0.14 |
| M5L | 2½ | 0.96 | 21.5 | 1.12 | 0.11 |
| M2R | 2¾ | 1.21 | 25.9 | 1.22 | 0.27 |
| M6L | 3 | 0.77 | 20.1 | 1.12 | 0.35 |
| M2L | 3½ | 1.19 | 25.4 | 1.05 | 0.30 |
| M7L | 3½ | 0.93 | 25.0 | 1.38 | 1.00 |
| M3R | 4½ | 0.78 | 25.0 | 1.44 | 1.04 |
| M3L | 5¼ | 0.80 | 27.3 | 1.86 | 4.23 |

Whether the alterations in elasticity and ductility which occur in a muscle with the onset of rigor mortis are due to changes in the myofibrils or in the sarcoplasm cannot be determined from the information now available. But it is evident that these changes in extensibility are due to alterations in the physical state of the muscle substance. What may be the chemical basis for these alterations in physical state also remains undetermined. Normally there is a great accumulation of lactic acid in a muscle preceding the onset of rigor mortis. But the work of Hoet and Marks (8) demonstrates that the accumulation of lactic acid is not the primary cause of the rigor. After the death of animals from thyroid overfeeding or fatal insulin intoxication the muscles go into rigor almost immediately after circulation stops and without the formation of much lactic acid. They obtained from 23 to 36 mgm. of lactic acid per 100 grams of tissue from muscles in rigor

following death from thyroid overfeeding and 54 mgm. from the muscles of an animal which had died from insulin overdosage.

Very similar alterations in elasticity and ductility are seen in living muscles in tetanus contracture. These will be discussed in another paper.

Muscle in potassium contracture is more ductile than muscle in rigor mortis or in tetanus contracture. That is to say, it is less able to maintain the shortened length when loaded with moderate weights. Figure 3 B is a tracing obtained from an isolated frog's gastrocnemius immersed in Ringer solution (NaCl—0.6 per cent; KCl—0.01 per cent; $CaCl_2$—0.01 per cent; $NaHCO_3$—0.01 per cent) and stretched in the manner already described with weights increasing by 20 gram intervals. At A in the same figure is shown the tracing obtained from a frog's gastrocnemius immersed

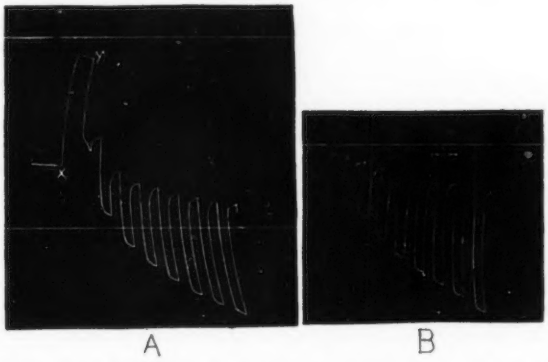


**Fig. 3.** Tracings obtained from the isolated gastrocnemius of the frog: A, potassium contracture, $x$ beginning of potassium contracture, $y$ the first loading; B, normal muscle.

in a solution containing 0.6 per cent KCl and 0.01 per cent $NaHCO_3$. While the muscle was still exposed to the air the unweighted lever was allowed to record its level on the drum. At $x$ the muscle was covered with the slightly alkaline solution of potassium chloride and the height of the contraction recorded. Such a muscle is able to support the weight of the lever for a considerable time but when the 10 gram scale pan was attached it pulled the muscle out almost to its original length, during which stretching, the after-lengthening was very pronounced. When the scale pan was lifted there was very little recovery chiefly in the form of an after shortening. When the scale pan containing 20 gram weight was supported from the lever it caused another large deformation, the muscle returning after the second unloading to a length slightly greater than its original length before treatment with KCl. Subsequent loadings in the series caused only slightly more deformation than in normal muscle.

The experiments which have been recorded here show that the elasticity and ductility of muscle are subject to wide variations. Muscle must be regarded as a viscous elastic body. To facilitate thinking one may picture it as "an elastic network containing a viscous fluid; and change of shape of the muscle would require the viscous fluid to pass more or less rapidly into a new configuration relative to the solid structures of the tissue." Gasser and Hill (2) from whom the preceding quotation was taken, have shown that the work done by a maximal tetanic contraction decreases as the speed of shortening increases, since a slower contraction allows more time for a rearrangement of the viscous elements. They found that an active muscle has a much higher coefficient of viscosity than a resting muscle, and is considerably less extensible.

In muscle contraction according to Fulton (9) "the contractile activity *per se* ceases at the angle, and the curve of relaxation is in reality no more than the curve of a viscous body stretched by a decremental force." Reasoning along these lines the slow relaxation of a muscle in fatigue contracture might be taken as an indication of an increased viscosity of the muscle. Even in a perfectly fresh muscle the terminal part of the relaxation is slow, approaching the base line in a curve that resembles the curve of the after-lengthening of a stretched muscle. Blix called attention to this resemblance, and, according to him, both the after-lengthening and the slow terminal part of the relaxation are due to the resistance which the muscle as a viscous body opposes to a change in its form.

The changes in ductility and in the secondary elastic phenomena reported in this paper can be interpreted along similar lines. It would seem that in rigor mortis at the same time that a muscle shortens its viscosity is increased. At first the viscosity is not so great but that the muscle can easily be pulled out by moderate loads, that is to say, the muscle is more ductile than normal. But in advanced rigor the viscosity has increased to a point which makes any internal arrangement very difficult, and as a result the ductility and secondary elastic phenomena are very greatly reduced. Later on, as the rigor passes off, we must assume that the viscosity again decreases enough to allow the muscle to be drawn out.

## SUMMARY

The primary and secondary elastic phenomena of muscle were studied in the living gastrocnemius of the white rat with intact circulation after section of the sciatic nerve, and in the gastrocnemius muscle of the white rat in rigor mortis. In the living atonic muscle the secondary elastic phenomena (after-lengthening and after-shortening) are not conspicuous and there is very little permanent deformation. In rigor mortis there is at first a marked increase in the after-lengthening, after-shortening and permanent deformation associated with a slight decrease in the primary

elastic stretch. Later as the muscle becomes more firmly set in rigor the primary and secondary elastic stretch are both greatly reduced and the permanent deformation becomes even less than in normal muscle.

In proportion as a muscle suffers permanent deformation or elongation when stretched it is plastic in the sense of Langelaan. But it is better to speak of this property as ductility rather than plasticity. Early in rigor mortis the ductility is increased, later it is decreased to less than that of living atonic muscle. But it increases again as the rigor begins to pass off.

A muscle in potassium contracture is very ductile and shows marked after-lengthening and after-shortening.

Ductility and the secondary elastic phenomena are probably dependent on the viscosity of the muscle which seems to be increased to varying extents in rigor mortis and potassium contracture.

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# THE ELASTICITY AND DUCTILITY OF MUSCLE IN THE MYOSTATIC CONTRACTURE CAUSED BY TETANUS TOXIN

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Received for publication June 25, 1928

By myostatic contracture we mean to designate a condition of more or less permanent shortening of resting muscle, which, after it has once developed, is quite independent of the nervous system. It persists under deep anesthesia or even after section of the motor nerve. If a limb is immobilized for many days in a flexed position, the flexor muscles shorten and become set at this new length so that when the restraining cast is removed the limb cannot be straightened. A similar type of shortening occurs after tenotomy and as a result of the unequal paralysis of antagonistic muscle groups in multiple neuritis and anterior poliomyelitis. It is surprising that practically nothing is known concerning the structural, physiological, or chemical alterations in muscle which are responsible for this type of shortening.

The shortening and setting of an immobilized muscle has been designated "Ruheversteifung" by Spiegel and Shiboya (1). They have clearly demonstrated that if the bony attachments of a skeletal muscle are immobilized for many days so as to prevent the frequent changes in length which normally result from spontaneous and reflex movements, there occurs a fixation of the muscle at the length thus imposed upon it. Fröhlich and Meyer (2) have shown that this contracture of an immobilized muscle is brought about through the central nervous system and does not develop in a denervated muscle. After removal of the left motor cortex from cats and fixation of both hind legs in plaster casts Spiegel and Shiboya found that contracture developed more quickly in the extensor muscles of the right and the flexor muscles of the left leg. This indicates that the contracture is an abnormal manifestation of tonic innervation.

In some preliminary experiments we found that 15 days after section of the tendo achillis the gastrocnemius muscle of the white rat had not only undergone shortening but it had become set at this new length and was able to support considerable loads without being drawn out to its original length. This was due to a change in the muscle itself because it persisted after section of the sciatic nerve. But in its genesis this contracture was dependent on nerve impulses, because it did not occur if the sciatic nerve was cut at the same time as the tendon.

When an appropriate dose of tetanus toxin is injected into one limb of a laboratory animal like the cat or rat the spasms develop only in the leg which received the injection. The extensor muscles shorten and the limb is held continuously in full extension. This persists for days without fatigue and without relaxation. In the early stages of local tetanus the limb relaxes when the animal is anesthetized, indicating that the shortened state of the extensor muscles is maintained by a continuous stream of nerve impulses reaching the muscles from the central nervous system. But in from 4 to 10 days after the injection, if the dose has been an adequate one, the muscles become set in this posture of rigid extension and fail to relax under anesthesia or even after section of the motor nerve. Tetanus contracture may persist for several days after section of the motor nerve in a muscle with intact circulation, the contracture disappearing gradually as atrophy begins (3, 4).

Until recently this type of contracture has received very little consideration because it was thought to be due to an overgrowth of the muscle with connective tissue. But a high grade tetanus contracture may develop in 5 days which is much too soon for fibrosis. Histological sections of such a muscle show very little that is abnormal aside from a slight blurring of the cross striations. There is no increase in connective tissue.

METHODS. In white rats weighing about 150 grams 0.05 cc. of tetanus toxin[1] was injected into the posterior part of the right thigh and distributed with a long hypodermic needle as widely as possible through the hamstring muscles. The toxin was not very strong and this dose was not sufficient to produce a general tetanus. By the end of the second day signs of local tetanus began to develop and on the third day the leg was held up off the ground and directed backward with the thigh and ankle extended and the knee flexed. In order to prevent the powerful quadriceps femoris from extending the knee, when a little later that muscle became involved in the spreading tetanus, the patellar tendon was cut 2 or 3 days after the injection. This permits the hamstrings to hold the knee flexed and prevents the stretching of the gastrocnemius muscle over an extended knee joint. The elasticity and ductility of the muscles were tested from 1 to 8 days after the tenotomy or from 3 to 11 days after the injection of the toxin.

Under urethane anesthesia the right gastrocnemius was prepared and stretched according to the manner described in the preceding paper. The circulation in the muscle was intact at the time of the stretching but the sciatic nerve was cut a few minutes before the stretching began. No attempt was made to obtain a control record from the left gastrocnemius because in earlier work (5) we had learned that the effect of the toxin

[1] A generous supply of this toxin was placed at our disposal by the Research Laboratories of Parke, Davis & Company.

spreads to the limb opposite to that which received the injection and it is therefore safer to use muscles from normal animals as a control.

After the extensibility of the right gastrocnemius had been tested the animal was killed and the left gastrocnemius prepared. Both muscles were then fixed under a tension of 25 grams in 10 per cent formalin for 24 hours or longer. Then the muscles were measured and weighed and the length of a definite easily identifiable bundle of parallel muscle fibers was determined.

TABLE 1

*Tetanus contracture in the gastrocnemius muscle of the white rat*

| NUMBER | DAYS AFTER INJECTION | DAYS AFTER TENOTOMY | LENGTH OF MUSCLE | | | LENGTH OF MUSCLE FIBERS | | WEIGHT OF RIGHT MUSCLE | STRETCH OF RIGHT MUSCLE | DEFORMATION OF RIGHT MUSCLE |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Difference | Right | Left | Right | Left | | | |
| | | | *mm.* | *mm.* | *mm.* | *mm.* | *mm.* | *grams* | *mm.* | *mm.* |
| T-25 | 5 | 0 | 1.1 | 24.0 | 25.1 | 8.3 | 8.9 | 0.94 | 3.00 | 0.97 |
| T-6 | 3 | 1 | 1.6 | 29.0 | 30.6 | 10.6 | 11.0 | 1.29 | 3.30 | 1.40 |
| T-26 | 3 | 1 | 2.1 | 25.9 | 28.0 | 9.7 | 10.0 | 1.02 | 3.43 | 1.09 |
| T-27 | 3 | 1 | 2.4 | 24.7 | 27.1 | 8.0 | 9.2 | 0.86 | 3.62 | 1.76 |
| T-17 | 6 | 3 | | | | | | | 2.28 | 0.90 |
| T-7 | 6 | 4 | 4.3 | 23.9 | 28.2 | 4.9 | 9.2 | 1.00 | 2.32 | 0.46 |
| T-9 | 6 | 4 | 4.3 | 23.8 | 28.1 | 5.0 | 9.0 | 1.02 | 2.22 | 0.60 |
| T-21 | 9 | 6 | 4.8 | 21.8 | 26.6 | 3.0 | 9.2 | 0.85 | 2.15 | 0.78 |
| T-11 | 6 | 4 | 4.9 | 25.1 | 30.0 | 4.1 | 10.0 | 1.13 | 2.34 | 0.65 |
| T-22 | 8 | 5 | 5.0 | 20.0 | 25.0 | 3.5 | 10.0 | 0.80 | 2.11 | 0.55 |
| T-14 | 6 | 3 | 5.2 | 21.7 | 26.9 | 4.0 | 9.8 | 0.78 | 2.14 | 0.66 |
| T-12 | 5 | 2 | 5.9 | 22.3 | 28.2 | 4.8 | 7.3 | 0.99 | 2.43 | 0.60 |
| T-13 | 5 | 2 | 5.9 | 24.0 | 29.9 | 5.0 | 9.3 | 1.00 | 2.20 | 0.60 |
| T-16 | 7 | 4 | 5.9 | 22.1 | 28.0 | 5.2 | 8.9 | 0.86 | 2.22 | 0.72 |
| T-23 | 11 | 8 | 6.1 | 22.2 | 28.3 | 3.2 | 8.0 | 0.80 | 2.00 | 0.46 |
| T-15 | 6 | 3 | 6.2 | 21.0 | 27.2 | 3.8 | 12.0 | 0.88 | 2.19 | 0.73 |
| T-20 | 7 | 4 | 6.7 | 22.2 | 28.9 | 3.4 | 10.0 | 0.95 | 2.05 | 0.50 |
| T-10 | 6 | 4 | 6.8 | 22.1 | 28.9 | 5.6 | 9.2 | 1.05 | 2.50 | 0.64 |

RESULTS. The measurements recorded in table 1 show that in every instance the gastrocnemius taken from the right leg which received the toxin was shorter than the corresponding muscle of the opposite leg. The difference in length between the two muscles varied from 1.1 mm. to 6.8 mm. according to the degree of the contracture developed in the right leg. This shortening of the right gastrocnemius had persisted after section of the sciatic nerve and after prolonged stretching of the still living muscle with weights increasing by 20 gram intervals up to 140 grams in the manner described in the preceding paper. This shows that a muscle in myostatic contracture can support considerable loads without being drawn out to its original length.

Since the amount of shortening is the best index of the degree of contracture we have arranged the data in the table in the order of magnitude of the difference in length between the two gastrocnemii. In the first four experiments on the list as thus arranged the muscles were certainly in an early stage of contracture, for not only was the time after injection and tenotomy short but the amount of shortening in the muscle was not great and there was little difference in the length of the muscle fibers in the gastrocnemii of the two sides. In each of the last 13 experiments listed in the table the muscle was in advanced contracture as evidenced by the marked shortening of the muscle fibers as well as of the muscle as a whole. There is no clear evidence of a gradation in the degree of the contracture within this group. The amounts of shortening in the muscle do not run parallel either with the length of time after injection and tenotomy or with the amount of shortening of the individual muscle fibers.

Fig. 1. Photograph of the gastrocnemii muscles of a white rat. The right muscle is in tetanus contracture; the control muscle is shown at the left.

The bundle of parallel muscle fibers which was selected for measurement showed a relatively much greater reduction in length than did the entire muscle. This is obvious from the table in spite of the very considerable variation in length of the fibers in the control muscles, a variation which is equally evident in perfectly normal muscles (5). In the last thirteen experiments listed in the table the average length of the fibers in the right gastrocnemius was 4.3 mm. as compared with an average length of 10.1 mm. for the fibers in the left or control muscle. The fibers in the right muscle were therefore reduced to less than half their original length. The much greater percentage of shortening in these fibers than in the muscle as a whole is to be accounted for by the oblique direction of the fibers within the muscle.

A photograph of the two gastrocnemii of rat 10 is reproduced in figure 1. The right muscle was shorter and plumper than the left but otherwise it had a normal appearance. When freshly exposed in the living animal its color was normal. It was not bound down by adhesions and presented no other abnormalities. What makes a muscle take such a shortened form and hold it after denervation even when subjected to a considerable longitudinal stress? When we are in position to answer this question we shall have learned something very fundamental about muscle physiology. An analogous condition is seen in the adductor muscle of molluses. Bethe

(6) found that the adductors of unfed and starving clams could hold the shell closed against a weight of 490 grams for at least 25 days. He calculated that striated mammalian muscle supporting the same weight by steady contraction for the same length of time would consume eight times as much sugar as the entire dry weight of the clam. The question naturally suggests itself whether myostatic contracture is in any way related to this shortened state of the molluscan adductor.

One thing is certain, the contracture is not due to fibrosis. Histological sections of such a muscle show no increase in connective tissue or other change which could account for the permanent shortening. An attempt is now being made to see if this can be accounted for on a chemical basis. It is generally believed that lactic acid formation is associated with and is perhaps the cause of the contractures produced by veratrine, acetylcholine and other drugs, and that an accumulation of lactic acid within the muscle is responsible for fatigue contracture. Cooper (7) has offered a similar explanation of the clinical varieties of contracture. Reasoning along similar probably fallacious lines, one might assume that in tetanus contracture there would be an accumulation of lactic acid in the muscle. This is, however, not the case. Doctor Davenport (8) has shown that the lactic acid content in these muscles is no greater than in the control muscle of the opposite side or in the muscles of normal animals.

The weight of the muscle was not altered. In a series of 25 rats with local tetanus the average weight of the right gastrocnemius was 0.969 gram and of the left 0.968 gram. The average weight of 10 muscles from normal rats was 0.989 gram. The rats used for this study were somewhat smaller than those used in a previous investigation, where the average weight of the gastrocnemius was found to be 1.364 grams in normal rats, and 1.338 grams on the side of the injection as against 1.410 on the control side of rats with local tetanus (5).

Although no structural or chemical alterations have yet been found which could account for the muscle assuming and holding a shorter than normal resting length, there are changes in the elasticity and ductility of the muscle which are clear evidence of a change in its physical state. These changes are indicated in the last two columns of the table and in figures 2 and 3. The upper two tracings in figure 2 were obtained from normal rat gastrocnemii by alternately loading and unloading with weights increasing by 20 gram intervals in the manner described in the preceding paper. The two lower tracings were obtained from the right gastrocnemii of rats 16 and 17 and show when compared with the normal tracings a marked decrease in elastic stretch and an increase in permanent deformation. The slightly more rounded angles and the somewhat more oblique short connecting lines indicate an increase in after-lengthening and after-shortening. These secondary elastic phenomena as they appear in normal muscle have

been discussed in some detail in the preceding paper. The decreased elastic stretch might be attributed to the shortening and thickening of the muscle fibers, but this would not account for the increased deformation and the more evident after-lengthening and after-shortening. The

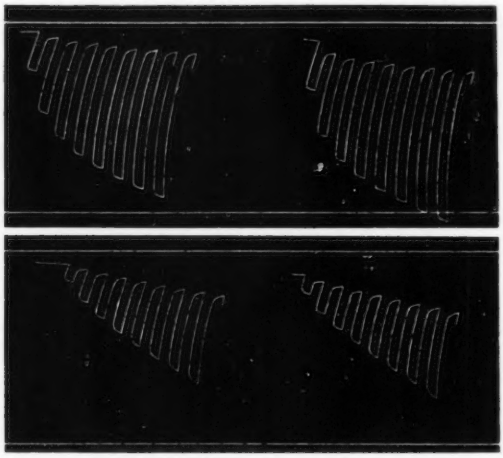

Fig. 2. Tracings showing the changes in the elasticity and ductility of muscle caused by tetanus contracture. The two upper tracings were obtained from normal muscles; the two lower ones were obtained from muscles in contracture (rats 16 and 17)

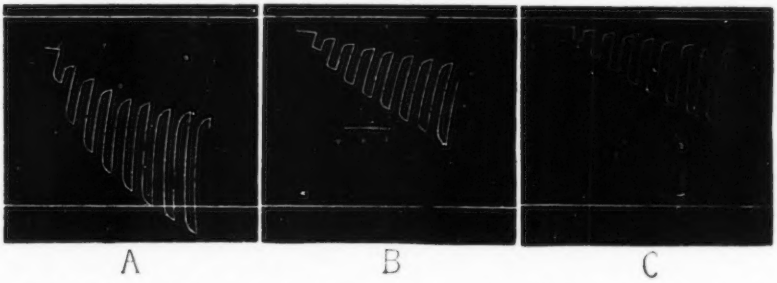


Fig. 3. Tracings showing the changes in elasticity and ductility of muscle at various stages of tetanus contracture: A, beginning contracture, rat T-6; B and C, advanced contracture, rats T-15 and T-20.

combination of decreased elastic stretch and increased ductility can only be explained on the basis of a change in the physical state of the myofibrils or sarcoplasm or of both.

At the time when the contracture is beginning there is only a slight

decrease in the elastic stretch, which is associated with a marked increase in the permanent deformation and in the after-lengthening and after-shortening (fig. 3 A). As the muscle becomes set more and more firmly in contracture the elastic stretch becomes still further reduced and at the same time the permanent deformation becomes less (fig. 3 B and C). In a series of 10 normal rats stretched in a similar manner the average permanent deformation amounted to 0.45 mm. In the 3 rats studied 3 days after the injection the deformation ranged from 1.09 mm. to 1.76 mm.; but at the height of the contracture 6 to 11 days after the injection the deformation was reduced to 0.5 or 0.6 mm. At the height of the contracture the elastic stretch at the final loading varied from 2 to 2.5 mm. as compared to an average of 3.8 mm. in the 10 normal rats.

There are many respects in which the myostatic contracture caused by tetanus toxin resembles rigor mortis. In both there is shortening of the muscle; and the alterations in elasticity and ductility are very similar. In both, there is at first a marked increase in ductility associated with a slight decrease in the elastic stretch. In both, as the muscles become more and more firmly set, the elastic stretch continues to decrease, while the ductility again decreases toward normal in tetanus contracture and to less than normal in rigor mortis. The two conditions, however, differ fundamentally in that in rigor mortis there is a great accumulation of lactic acid in the muscle, while in tetanus contracture the lactic acid content remains normal (8). But this difference is perhaps not as important as it seems, because after thyroid overfeeding rigor develops immediately after death with very little lactic acid in the muscles (9). Lactic acid is therefore not the cause but a concomitant of the rigor. We do not wish to overstress the resemblance between the rigor of dead muscle and myostatic contracture which develops in living muscle with intact circulation. Nevertheless both seem to be due to a somewhat similar alteration in the physical state of the protoplasm of the muscle fibers.

## SUMMARY

Myostatic contracture is a condition of permanent shortening in resting muscle which persists after section of the motor nerve. It develops whenever a muscle is immobilized by section of its tendon, by paralysis of its antagonists or by fixation of the limb in a plaster cast. It also develops in the late stages of experimental local tetanus.

A study of the gastrocnemius muscle of the white rat in myostatic contracture due to tetanus toxin showed not only a permanent shortening of the resting muscle but also an even greater relative shortening of the individual muscle fibers. The elastic stretch of a muscle in tetanus contracture is less for a given load than would be the stretch of a normal muscle under the same conditions. In the early stages of tetanus contracture,

the after-lengthening, after-shortening and ductility are markedly increased; but they decrease again toward normal as the muscle becomes more rigidly set in contracture.

The extensibility of a living muscle in myostatic contracture caused by tetanus toxin resembles that of a muscle in rigor mortis. In both the changes in elasticity and ductility are probably related to similar changes of the physical state of the muscle fibers.

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# THE PENETRATION OF ULTRAVIOLET RAYS INTO LIVE ANIMAL TISSUE

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Received for publication June 27, 1928

A previous paper by Macht, Anderson and Bell (1) reported results in the quantitative measurement of ultraviolet transmitted by living rabbit skin 1.175 mm. thick. These measurements, made with the aid of a large quartz prism monochromator and a bismuth silver thermopile, indicated transmission of the shorter ultraviolet to a remarkably high degree.

Spectrograms of transmission through living rabbits, cats and human flesh fresh from the operating room which were included in this same report indicated no transmission shorter than 2800 Angstrom units, and were apparently inconsistent with the quantitative measurements.

The data included in that paper have been the subject of much comment and criticism (2), (3), (4) and as a result the extent of penetration of ultraviolet has become an even more debated subject than before.

The authors have made very extensive investigations of the methods employed in these experiments and have repeated their transmission measurements using a very much refined technic and every conceivable precaution. The results which are included in this paper should be a definite and final answer to all who are interested in this subject.

The writers find their quantitative measurements of ultraviolet transmission as determined by the thermopile and published in the J. A. M. A. on January 21, 1928, were not accurate; too great a transmission was shown in the shorter wavelengths.

Bachem (2) has suggested that "a window glass inserted in the beam before the thermopile would apparently transmit a magnitude of about 50 per cent at about 2800 Angstrom units, although in reality it should not transmit any trace of light." During this experiment, a plate of window glass inserted between the monochromator and rabbit for wavelengths 2800, 2650 and 2537 resulted in an immeasurable thermopile reading;

[1] Assisted by Messrs. Bird, Fraser, Kamphaus and Kopf.

indicating that the quantity of stray and scattered ultraviolet, visible and infra red light were negligible and not introducing any appreciable error. However, error did exist and would have been detected if the glass plate had been placed between the thermopile and rabbit instead of between the monochromator and rabbit. The absorbed ultraviolet causes the skin to fluoresce brilliantly. This fluorescent light was collected by the lens system and focused on the thermopile together with any transmitted light, the thermopile reading being a composite of both. The extent of fluorescence increased markedly with shorter wavelengths, and therefore introduced relatively greater error in this portion of the spectrum. However, in spite of these errors introduced by a failure to appreciate the influence of fluorescence, there exists measurable transmission at 2537 Angstrom units as subsequent experiments have shown.

The spectrograms made through the live skin of a living rabbit (see B and D, fig. 3) (1) terminate at 2967 Angstrom units because the exposure time, 1 minute, was not sufficiently long to enable the shorter and weaker bands to register. The procedure in the present investigation eliminated this difficulty.

*New experiments on transmission. Preparation of animal.* The animal employed was a greyish-yellow rabbit, weight 1.8 kgm. The skin had a few pigmented spots. Paraldehyde anesthesia assisted by occasional applications of ether, was used throughout the experiment which lasted for eight hours.

The skin of the abdomen was carefully shaved, and a layer 1.2 mm. thick partially detached, the operation being performed in such a way that there was no bleeding. The skin layer was kept moist with a physiological sodium chloride solution. This solution is nearly perfectly transparent to ultraviolet radiations to 2000 Angstrom units.

The rabbit was fully alive throughout the experiment, and was killed with ether afterwards. The skin segment through which the measurements were made was alive and reacted to cold and touch until nearly the end of the experiment. Spectroscopic measurements, subsequently shown, clearly indicated that progressive changes were occurring in the skin layer, resulting in changing transmission.

*Method of quantitative measurement.* Fluorescent photometry was employed for the quantitative measurement of the ultraviolet light instead of the thermopile.

Uranium glass which responds with brilliant fluorescence to all wavelengths of the ultraviolet, violet and blue served as the receiver. This glass does not fatigue or change in any way through the action of the light, and does not respond to green, yellow, red and infra red radiations. The receiver was a block about $1\frac{1}{2}$ inch square with one edge bevelled to $45^\circ$ and rough ground. This arrangement resulted in complete absorption of the exciting radiations.

The photometer was arranged in the usual form employing the inversed square law of illumination. A frosted 100 watt nitrogen filled lamp was mounted on a car whose motion was guided by a track. A diaphragm located immediately in front of the lamp reduced the intensity of light to that needed for these experiments and because of the smallness of the opening, produced a very nearly point source of light. The entire apparatus was arranged so that the illumination of the comparison screen of the photometer varied inversely as the square of the distance to the diaphragm.

The comparison screen was a block of opal glass, and together with an especially prepared plate of fluorescent uranium glass formed a photometer head arranged in such a way that the white light from the opal glass and the fluorescent light could be viewed side by side with an almost invisible edge between them. To the eye, the light from the illuminated screen and from the uranium glass appeared as two elongated rectangles side by side and of equal size. The fluorescent light was of a green-yellow color, and in order to make accurate comparisons, it was necessary to view both this light and the white light from the opal glass through a monochromatic green light filter, so that both sources of light appeared to the eye to be of the same color. Owing to the high sensitivity of the eye to green light, balances could be made with very good accuracy.

*Monochromatic ultraviolet.* The apparatus and arrangement for producing monochromatic ultraviolet light was similar to that employed in the previous experiment (fig. 5) (1), with the exception that the quartz mercury arc was in direct contact with the primary slit. Slit dimensions were maintained at 1.2 × 44 mm. throughout the entire experiment. The optical system was constructed of crystal quartz of the two polar varieties. The lenses were 75 mm. in diameter, the prism 60 × 80 mm. of the double cornu type. The spectralized light was brought to focus on a secondary slit, the length of the spectrum produced extending from 1850 A.U. to red, a distance of 90 mm. The entire spectrum could be easily and definitely discerned by the interposition of a fluorescent uranium plate before the slit. The desired ultraviolet band could be isolated by rotating the prism until the band fell upon the slit and adjusting the focusing lens. The slit dimensions employed for these experiments were 1 × 44 mm.

The purity of the monochromatic light is definitely shown in subsequent spectrograms and stray radiations have been measured quantitatively.

The intensity of the lamp did not vary more than 5 per cent during the experiment.

*Calibration of photometer and stray light.* For purely relative measurements, such as the transmission of ultraviolet through rabbit skin, it is not essential that the photometer be calibrated, that is, the response to the various wavelengths be known. The actual measurement involved consists in 1, measurement of intensity of band; 2, measurement of in-

tensity of band through tissue. The ratio of the two, with any necessary corrections, represents the transmission.

However, we have chosen to calibrate the photometer against the thermopile, since such a procedure would establish greater confidence in those who are not familiar with photometric procedure.

*Calibration 1.* The relative intensities of six monochromatic ultraviolet bands and the violet band were measured. The photometer head was placed alongside of the thermopile, both units being mounted on a little car, the arrangement being such that either the fluorescent receiver or the pile units could be brought into the path of the monochromatic light. Alternate readings of photometer and galvanometer were made, the photometer readings being in inches, the galvanometer in millimeters. The results obtained were calculated in terms of relative intensity, the

TABLE 1

*Agreement between fluorescent photometer and thermopile measurement of monochromatic bands of quartz mercury arc*

| WAVELENGTH | THERMOPILE | | PHOTOMETER | |
|---|---|---|---|---|
| | Galvanometer deflections | Relative intensity | Distance squared | Relative intensity |
| *A.U.* | *mm.* | | | |
| 4,045 | 122 | 26 | 32 | 26 |
| 3,660 | 461 | *100* | 125 | *100* |
| 3,130 | 301 | 65 | 83 | 66 |
| 3,025 | 156 | 34 | 42 | 34 |
| 2,800 | 55 | 12 | 15 | 12 |
| 2,650 | 102 | 22 | 26 | 21 |
| 2,537 | 115 | 25 | 31 | 25 |

intensity at 3660 being arbitrarily designated as 100. Table 1 shows the remarkable agreement between the two methods, and definitely indicates the freedom of the monochromatic light from stray visible and infra red radiations, for the thermopile responds readily to these radiations whereas the photometer is not affected in any manner.

*Calibration 2.* A further check on the purity of the monochromatic light was made by the measurement of the transmission through Corning purple Corex (G986A) (4 mm.) and window glass (2 mm.)

Purple Corex is an admirable substance for indicating the amount of stray ultraviolet radiations present in a measurement of weak intensities of the shorter wavelengths, for the glass transmits the usual impurities, 3660 and 3130 to a high degree and 2650 and 2537 to only a slight extent. Thus a measurement of the shorter wavelengths through this glass decreases the intensity of short wavelength, but transmits about 75 per cent

of the stray ultraviolet. Such measurements serve to indicate the amount of stray long waved ultraviolet contaminating measurements of short ultraviolet.

A spectrogram of the window glass showed no transmission shorter than 2967 Angstrom units, and an over-exposure was required to register this wavelength. Consequently, any light energy measured when short wavelengths are incident on the window glass must be "stray light."

Table 2 gives the percentage transmission of these glasses determined both radiometrically and photometrically.

The results obtained with purple Corex show that the "stray" ultraviolet present when 2537 and 2480 are measured, supposing all the transmitted light to be stray light, cannot possibly be greater than 3 and 0.7 per cent respectively. Since it is definitely known that 2537 and 2480

TABLE 2

*Measurement of "stray light" (Percentage transmission of glasses)*

| WAVELENGTH | PURPLE COREX (G986A) | | WINDOW GLASS | |
|---|---|---|---|---|
| | Thermopile | Photometer | Thermopile | Photometer |
| *A.U.* | | | | |
| 4,045 | 2.5 | | 90.9 | |
| 3,660 | 75.0 | 71.5 | 86.3 | 86.3 |
| 3,340 | 78.0 | 80.0 | 38.0 | 35.8 |
| 3,130 | 74.0 | 75.0 | 0.9 | 1.09 |
| 3,025 | 66.0 | 65.0 | 0 | 0.054 |
| 2,967 | 58.0 | 57.0 | 0 | 0.050 |
| 2,800 | 35.0 | 33.0 | 0 | 0.045 |
| 2,650 | 14.0 | 15.0 | 0 | 0.045 |
| 2,537 | 2.5 | 2.55 | 0 | 0.040 |
| 2,480 | 0.5 | 0.49 | 0 | 0.040 |

are transmitted by this glass, it is evident that the percentage of impurity must be less than these figures.

The thermopile was not sufficiently sensitive to measure any radiations through the window glass when wavelengths shorter than 3130 Angstrom units were employed. However, the photometer, which is considerably more sensitive, did measure radiations throughout the ultraviolet. Since the glass did not transmit shorter than 2967, any transmission measurement for shorter wavelengths must be due to "stray" long ultraviolet, violet and blue light. Green, yellow, red and infra red radiations as previously stated, do not register on the photometer. The "stray" light transmitted by the glass amounted to 0.04 per cent of the incident light, and was therefore negligible.

The above measurements and the spectrum plates on the following pages

should be sufficient evidence to satisfy any queries as to the purity of the monochromatic light.

*Influence of fluorescent radiation from rabbit skin.* The earlier experiments were in error because the fluorescent light from the rabbit skin was focused, together with the transmitted light, on the thermopile. In the case of the shorter wavelengths, the fluorescent light actually exceeded the ultraviolet transmitted light in intensity, causing a very large error.

The skin begins to fluoresce at 3660, the color of the light emitted at this wavelength being distinctly blue. The fluorescence continues throughout the range of ultraviolet studied, the color becoming more whitish with the shorter wavelengths. The fluorescent light is completely cut off by purple Corex, and therefore appears to be devoid of ultraviolet. With a visual spectroscope, the light appears to consist of blue, green, yellow and red, the blue predominating.

Blue light affects the photometer, so it was necessary to arrange the lens system in such a way that the light transmitted through the rabbit skin was focused on the photometer receiver and the fluorescent light was scattered. This could be arranged, because whereas the second slit served as the light source for the band of monochromatic light, the rabbit was the source of fluorescent light, the difference in position resulting in different focusing conditions.

The lens arrangement appeared to be absolutely satisfactory, for when the skin was irradiated by 2800, 2650 or 2537, the insertion of a glass plate between the skin and photometer completely blotted out every trace of fluorescent light in the photometer. Since the photometer can measure transmission to 0.01 per cent for the strong bands, any stray light through the animal tissue, or action by fluorescent light from the animal skin must have been still smaller.

*Quantitative transmission of monochromatic ultraviolet through live skin 1.2 mm. thick of a living rabbit.* The apparatus, animal, procedures and precautions described above were employed for measuring the transmission of monochromatic ultraviolet radiations through skin tissue 1.2 mm. thick. The area of skin tissue irradiated was 7 × 12 mm. All the incident radiation was either reflected, absorbed or transmitted. The skin was *not* dehematized. Its surface was washed frequently with a physiological sodium chloride solution to prevent dehydration.

The photometric measurements of transmission were made by three observers. The photometer was unset after each reading so that the new observer had not the faintest conception as to where it had been set for the preceding observation. The scale could not be seen by the observer. These precautions resulted in three absolutely independent measurements of the transmitted light, the results of which are included in table 3.

The results cannot be dismissed on the basis of "stray" light. Where

the rabbit was irradiated with 3025, 2800, 2650 and 2537 a plate of window glass inserted between the rabbit and the photometer receiver completely blocked out the light which was producing fluorescence of the uranium glass receiver. The photometric measurements give irrefutable evidence of an appreciable transmission of the short ultraviolet through living skin tissue.

Quantitative measurements were completed within two hours after operation on animal.

TABLE 3

*Percentage transmission of monochromatic ultraviolet light through live rabbit skin of living rabbit, skin thickness 1.2 mm.*

| WAVELENGTH | OBSERVER | | | AVERAGE |
|---|---|---|---|---|
| | Bird | Fraser | Anderson | |
| *A.U.* | | | | |
| 4,045 | 16.0 | 16.0 | 16.5 | 16.0 |
| 3,660 | 15.0 | 14.0 | 13.0 | 14.0 |
| 3,130 | 8.4 | 10.7 | 9.2 | 9.4 |
| 3,025 | 7.5 | 6.6 | 8.4 | 7.5 |
| 2,800 | 10.1 | 9.4 | 9.8 | 9.8 |
| 2,650 | 7.3 | | 7.4 | 7.4 |
| 2,537 | 6.3 | 7.1 | 6.3 | 6.6 |

TABLE 4

*Exposure time for production of monochromatic spectrograms of equal intensity for 100 per cent transmission*

| WAVELENGTH | RELATIVE INTENSITY | TIME (IN SECONDS) | |
|---|---|---|---|
| | | Without rabbit (Plate A) | Through rabbit (Plate B) |
| 4,045 | 26 | 4.0 | 24 |
| 3,660 | 100 | 1.0 | 6 |
| 3,130 | 66 | 1.5 | 9 |
| 3,025 | 34 | 3.0 | 18 |
| 2,800 | 12 | 8.0 | 48 |
| 2,650 | 21 | 5.0 | 30 |
| 2,537 | 25 | 4.0 | 24 |

*Qualitative transmission of ultraviolet through live rabbit's skin 1.2 mm. thick.* The qualitative measurements were made with the aid of a Hilger quartz spectrograph employing Cramer Isochromatic medium emulsion dry plates. A slit width of 0.25 mm. was employed, the slit being placed in the position occupied by the photometer receiver during the quantitative measurements.

The exposure time was made proportional to the intensity of the monochromatic bands without the rabbit skin interposed, and the actual time

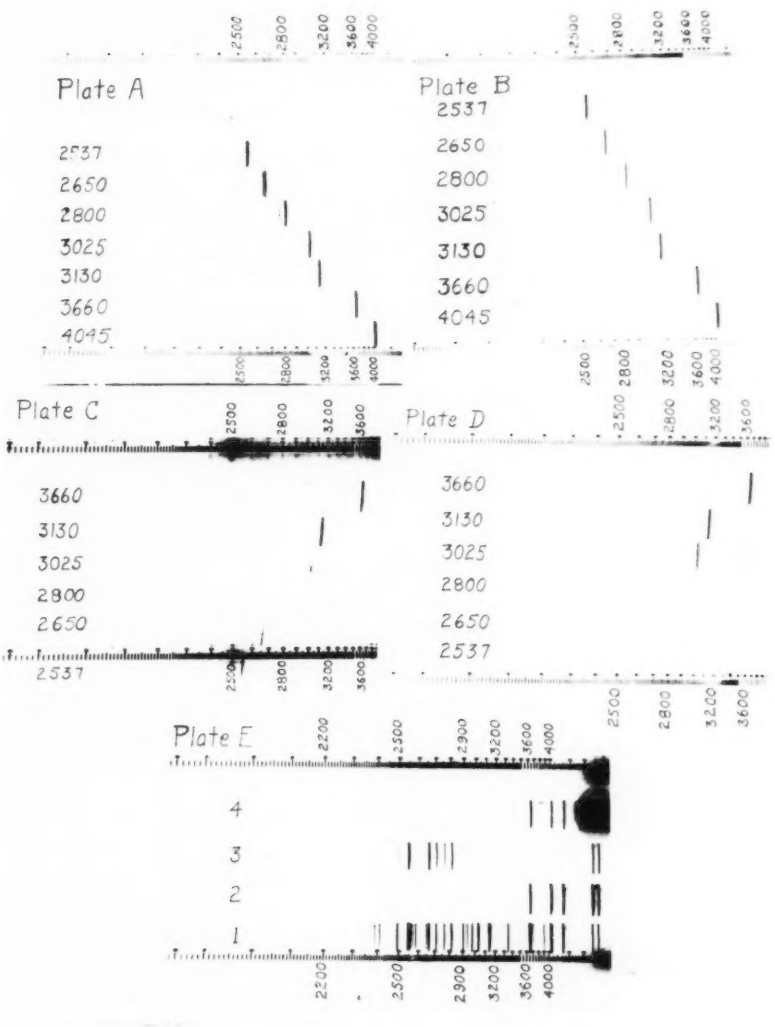
Plate A
2537
2650
2800
3025
3130
3660
4045
Plate B
2537
2650
2800
3025
3130
3660
4045
Plate C
3660
3130
3025
2800
2650
2537
Plate D
3660
3130
3025
2800
2650
2537
Plate E
4
3
2
1

Fig. 1

of exposure was a multiple of this, being based on the intensity of the violet band (4045 A. U) with and without the rabbit skin. Table 4 gives the exposure times for plates A and B.

Plate A. Monochromatic bands without rabbit.

Plate B. Monochromatic bands through rabbit skin 1.2 mm., 2 hours after operation on animal.

Plate C. Monochromatic bands through rabbit skin 1.2 mm., 4 hours after operation on animal.

Plate D. Monochromatic bands through rabbit skin 1.2 mm., 6 hours after operation on animal.

Plate A shows clearly the monochromatic character of the light, and serves as a basis of comparison for the succeeding plates.

Plate B, taken two hours after the operation on the animal shows definitely the transmission of the ultraviolet through the living skin tissue. The exposure time for 4045 was such that the photographic plate received approximately the same quantity of light for both plates A and B. The time for the other bands was proportional to the intensity of the light incident on the rabbit skin, and not to that of the transmitted light (table 4).

Plate C taken 4 hours after the operation on the animal, had to be retaken. The first exposure employed the same time ratios as were used for plate B. When the plate was developed the only lines that appeared were 3660, 3130 and 3025 (4045 was not taken). A new plate was made, the exposure time for 2800 being increased to 360 seconds, that for 2650 to 240 seconds, for 2537, 240 seconds. Even with these lengthy exposures, as shown in plate C, 2800 did not register, while 2650 and 2537 have just about appeared. The extremely small quantity of these short wavelengths that was present when this plate was taken is shown by comparison with "stray" light which was beginning to register on the plate. It is very evident that during the 2 hours elapsing between the taking of plates B and C, there had been changes in the rabbit's skin tissue resulting in decreased transparency to the short wavelengths.

Plate D was taken six hours after the animal had been operated on. The skin at this time was beginning to appear moribund. The exposure time was twice that finally employed for plate C. The spectrogram shows not the slightest trace of any radiation shorter than 3025. The disappearance of the "stray" light, and the decreased intensity of the longer wavelengths in view of a doubled time of exposure indicate still further changes in the rabbit's skin.

Additional spectrum plates were taken, but are not included as they are similar in every way to plate D.

*Transmission of ultraviolet through dead rabbit skin 1.2 mm. thick.* After the rabbit had been killed with ether, the piece of skin was detached and preserved in a physiological sodium chloride solution until the following day, when the qualitative transmission was determined by spectrograms employing the full light from the quartz lamp instead of that from the monochromator.

| Plate E | *seconds* |
|---|---|
| 1. Spectrum quartz mercury arc | 0.25 |
| 2. Through dead skin 1.2 mm. thick | 60 |
| 3. Quartz mercury arc through Cl-Br gas filter | 1 |
| 4. Through dead skin and gas filter | 600 |

Plate E indicates that the dead skin had negligible transmission for wavelengths shorter than 3660. The transmission of 3660 is very small when it is considered that the exposure time through and not through the skin bears the ratio of 240 to 1.

The chlorine-bromine gas filter was employed in order to enable a long exposure so that it could be determined whether even a trace of the short ultraviolet could penetrate the dead tissue. An exposure of 600 seconds indicates not a trace of radiation in the region to which the gas filter is most transparent. The long ultraviolet which registered is due to "stray" light through the gas cell,[2] combined with a long exposure.

The measurements indicate that the dead skin, 1.2 mm. thick, is practically opaque to ultraviolet shorter than 3660 A. U.

CONCLUSIONS. The experiments described in the preceding pages definitely establish, quantitatively and qualitatively the transmission of the short ultraviolet through live animal tissue, between 6 to 10 per cent of the short ultraviolet 3000 to 2537 penetrating through a thickness of 1.2 mm.

Whether this shorter ultraviolet can produce biological reactions after it has penetrated the tissue is a problem for the biologist. Experiments by Hess and Anderson (5) have definitely demonstrated the therapeutic effectiveness in rickets of the short ultraviolet employing only radiations of wavelength 2650 to 2800 inclusive.

These experiments serve as an indication of the possible effectiveness of these short wavelenths in other biological reactions. Further experimentation is necessary, but it must not be performed through tissue which is not fully alive, for our spectrographic data show conclusively the great difference between fully alive, partly alive and dead tissue. Experiments such as described by Leonard Hill and others (3), (6) in which dead skin was used give absolutely no suggestion as to what would actually occur if the skin were alive. The entire subject of biologic light action will be greatly clarified when biologists, physiologists, investigators and practitioners in general realize the difference in behavior of *live* skin as opposed to *dead* skin.

## SUMMARY

1. Measurements of penetration of ultraviolet rays through living animal tissue employing fluorescent photometry, spectroscopy and every conceivable precaution, have demonstrated beyond all doubt the marked transmission of short ultraviolet rays.

2. Certain errors have been shown to have existed in penetration measurements previously reported by Macht, Anderson and Bell. These errors have not in any way invalidated their conclusions.

3. Transmission for a thickness of 1.2 mm. was found to be 6 to 10 per cent for wavelengths 2537 to 3000 Angstrom units.

[2] (The gas cell transmits about 0.1 per cent of the long ultraviolet.)

4. Spectrograms confirmed photometric results.

5. Spectrograms taken at intervals definitely establish progressive changes in transmission which occur while skin is dying.

6. Importance of studying biologic light reactions through live skin is emphasized.

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# NOTES ON THE ARRANGEMENT OF BLOOD VESSELS WITHIN THE FROG'S KIDNEY TOGETHER WITH SOME MEASUREMENTS OF BLOOD PRESSURE IN THE RENAL PORTAL AND RENAL VEINS

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Received for publication June 21, 1928

The usefulness of the frog's kidney in certain studies of renal physiology makes an accurate knowledge of its blood vessels of importance. Appreciation of this led Nussbaum (1886) to present a detailed and careful description of the vascular channels in *Rana esculenta* and *Triton* as part of his studies of kidney function. Investigations in progress in this laboratory have made more detailed knowledge of certain questions of the finer vascular anatomy of the frog's kidney seem desirable. These queries include the possibility of overlapping in the distribution of adjacent renal arteries, the presence and number of arteriae rectae, the approximate number of glomeruli, the character of the renal portal-renal vein anastomosis, and the blood pressure in the various renal vessels.

*Arterial distribution.* The arterial distribution has been studied in twenty-one animals by means of corrosion preparations after injection of celloidin, and in histological sections after injection of soluble Berlin blue. The technique described by Hinman, Morison and Lee-Brown (1923) was followed. *R. pipiens* or *catesbiana* was pithed, a cannula inserted into the aorta just above its bifurcation and the aorta tied immediately below the origin of the coeliaco-mesenteric artery. The kidneys were washed free of blood with physiological saline solution before injection.

The origin and distribution of the urogenital arteries in these preparations agreed in the main with the descriptions given by Nussbaum (1886) and by Ecker and Wiedersheim (1896). The urogenital arteries, from four to seven to each kidney, arise from the ventral surface of the aorta, either as single unpaired vessels, which soon divide into right and left branches, or as separate vessels to each kidney. A variable number of small muscular arteries arise from the aorta just dorsal to the origins of the urogenital arteries; less frequently such vessels branch from a urogenital artery itself. A typical urogenital artery then divides into a renal artery and a genital artery, the latter being much smaller in the male than in the female. The first urogenital artery supplies branches to the fat

bodies. The lowest artery to each kidney constantly arises from a common unpaired vessel of unusual length, gives no genital branch, and supplies the whole lower pole of the kidney. A renal artery, after division from the genital, arches dorsally and caudalward, giving off numerous branches which again divide one or more times to end as afferent glomerular arteries.

There is considerable overlapping in the distribution of the renal arteries, so that the same area of kidney is frequently supplied by more than one vessel. This was shown in celloidin preparations hardened *in situ* (fig. 1, a and b). The number of afferent arterioles in many areas was about equally divided in their origin between adjacent renal arteries. In some preparations a primary division of one renal artery transgressed the territory mainly supplied by the next lower renal artery. Additional evidence of overlapping was obtained in preparations in which the aorta was ligated between two urogenital arteries and a gelatin mass of one color injected into the aorta above the kidneys and a different colored mass into the aorta below. The position of red and blue glomeruli in horizontal serial sections was then sketched with a camera lucida (fig. 2). There was no sharp line of division between the territories supplied by different renal arteries. It seems evident that it must be extremely difficult to be sure that the arterial blood supply of all the glomeruli belonging to the tubules in a particular area of the kidney has been interrupted by ligating particular renal arteries. This overlapping does not apply to the lower pole of the kidney, supplied by the lowest renal artery. In its course across the kidney a genital artery not infrequently gives off small branches which enter the kidney substance to end as afferent arteries of unusual length. The length of the afferent arteries varies from about 0.09 to about 0.7 mm., most being approximately 0.1 to 0.2 mm. The glomeruli in the lateral portion of the kidney are usually supplied by the longer afferent vessels.

Occasionally a small artery breaks up immediately into capillaries, as described by Nussbaum (fig. 1c). The number of such arteriae rectae, however, is small, one or two being found in any preparation, while in a number of preparations none were detected. This is in agreement with the observations of Huber (1906), Traut (1923) and Morison (1926) on the mammalian kidney.

The first urogenital artery frequently arises immediately below the arteria coeliaco-mesenterica, at some distance above the upper pole of the kidney. This has frequently led to embarrassment in attempts to ligate the renal arterial supply. Attempts to demonstrate anastomoses between branches of the arteria coeliaco-mesenterica and renal arteries were unsuccessful. When the aorta was ligated above the first urogenital artery, injections of dye into an aortic arch uniformly failed to stain the kidneys, provided the intestinal tract was ligated at or above the cloaca. The anastomoses of the coeliaco-mesenteric artery with the art. hemor-

rhoidalis, and so with the renal portal system, described by Nussbaum, was confirmed.

On entering a Malpighian body an afferent artery usually divides into three primary divisions, each of which then divides again at least once. This secondary division is quite irregular, so that the lengths of unbranched capillaries varies greatly. Of further division of the glomerular capillaries we have not sufficient data for presentation. Satisfactory evidence of anastomoses between capillaries derived from different primary divisions of the afferent vessel was not obtained. This is in agreement with Vimtrup's (1928) study of the mammalian glomerulus. The number and size of injected capillaries varied considerably in different glomeruli. This confirms Richards and Schmidt's (1924) observations of the living frog.

The number of glomeruli in each kidney, counted after staining with Janus green (Nelson 1922) ranged from 1592 to 5260 in seventeen kidneys,[1] most frequently being about 2000. The glomeruli are chiefly in the middle zone of the thicker medial part of the kidney. The thinner lateral margin, however, contains a considerable number. The diameter of the glomeruli in corrosion preparations varied from 93 to 162 micra in diameter. This is about one half the size in the living animal measured by Richards and Schmidt.

The efferent glomerular vessels differ in length, as do the afferent vessels. The efferent vessel joins the peritubular capillary plexus. We have not encountered unequivocal evidence of an afferent vessel joining a branch of renal vein or renal-portal vein directly as pictured by Nussbaum. The intertubular plexuses are so distensible that it is difficult to distinguish union of an efferent vessel and distended intertubular sinus from a possible anastomosis with a true vein.

*Distribution of renal portal vein.* Nussbaum, from injections and from observations of the living Triton kidney, described a complete mixing in the tubular capillary network of blood brought to the kidney by the renal-portal vein and that derived from the efferent glomerular vessels and arteriae rectae. This conception is commonly accepted. Woodland (1922), however, from experiments on the large Indian frog *R. tigrina*, came to the conclusion that the intertubular capillary plexus is structurally and physiologically distinct from the renal-portal meshwork, and that little of the blood brought to the kidney by the renal-portal vein comes into intimate contact with the tubules. He diagrams large channels connecting the renal afferent vein with the caval system. More recently Smith (1927) has maintained that neither Nussbaum's nor Woodland's conception is correct, but that "the blood vessels from the renal-portal veins in the frog do not break up into separate capillary beds and supply the

[1] Several of these counts were made by Doctor Walker in the course of another study.

tubules or any other portion of the kidney with blood, but pass through the kidneys in relatively large channels which anastomose with the renal arterial system." Smith's diagram of Nussbaum's conception is incorrect, and does not correspond with Nussbaum's picture. Bensley and Steen (1928) have called attention to this error.

Information on the nature of this venous meshwork is necessary for an interpretation of the Nussbaum experiment or modifications of it. We have been unable to obtain any evidence of direct communication or large vessels between renal-portal vein and renal vein in eighteen frogs. Corrosion preparations after injection of colloidin into the renal portal vein show free communication with the branches of the renal vein, but always by many small anastomosing channels. When a solution of Berlin blue is injected gently via the renal portal vein into a previously washed kidney observed under the microscope, the dye does not pass directly into a branch of the renal vein, but spreads out through tortuous intertubular channels before reaching a tributary of the renal vein. The same phenomenon was observed in another experiment designed to maintain closely the normal pressure in the renal portal system. A small frog was pithed, the anterior abdominal vein doubly ligated and cut near its cephalic end, and a flap made from abdominal wall containing the vein. The vein was then clamped above the pelvis and the free segment filled with a half-saturated solution of trypan blue. While observing the kidney under the microscope the clamp on the anterior abdominal vein was removed and the flap gently elevated, allowing the dye to run in by gravity at a pressure just in excess of that in the pelvic veins. A blue color was promptly visible in the renal portal vein, then a diffuse blue pervaded the kidney mass. Only after this could color be seen in the renal vein.

When a 1 per cent solution of soluble Berlin blue is perfused through the aorta at 40 to 46 cm. $H_2O$ pressure and 1.8 per cent colloidal lead chromate through the renal portal vein at an appropriate pressure (5 to 7 cm. $H_2O$) the distribution of the two pigments in the intertubular capillaries in histological sections is indistinguishable. Some capillaries may contain blue only, some yellow, others an intimate mixture of both. Each pigment is in intimate contact with the tubular cells. Such perfusions afforded no evidence of separate systems of vessels, described by Woodland, but indicate the free anastomoses pictured by Nussbaum.

In these sections blue pigment was commonly present in the glomerular capillaries, in the lumen of some tubules, and occasionally within the tubular epithelium. A few glomeruli contained granules of lead chromate in their capillary loops—a demonstration of the accessibility of glomeruli to renal portal perfusion fluid pointed out by Richards and Walker (1927).

We have been unable in many attempts to confirm Smith's observation of the direct and easy passage of small air bubbles or globules of mercury

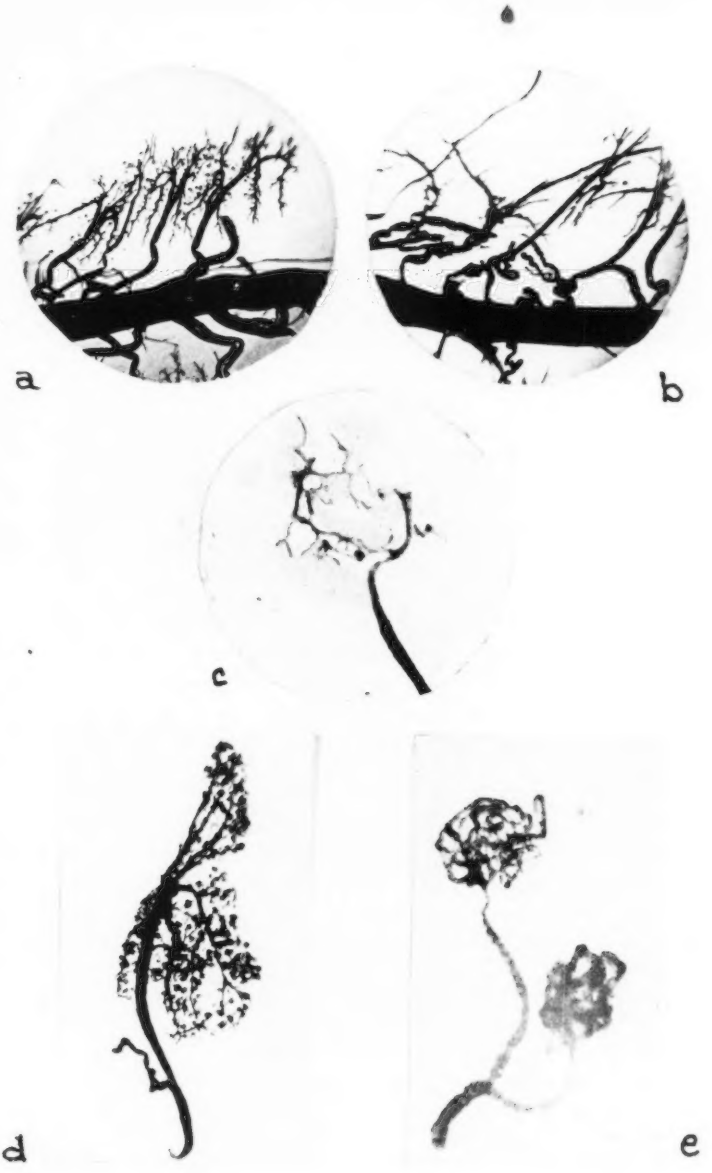



Fig. 1. Corrosion preparations of blood vessels in the frog's kidney: *a* and *b* to show overlapping in distribution of renal arteries (×8); *c* an arteria recta (×54); *d* completely injected renal artery (×27); *e*, two glomeruli isolated by dissection (×100).

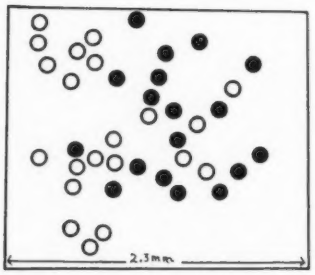



Fig. 2

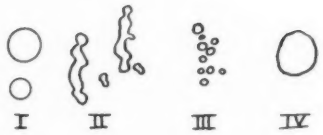
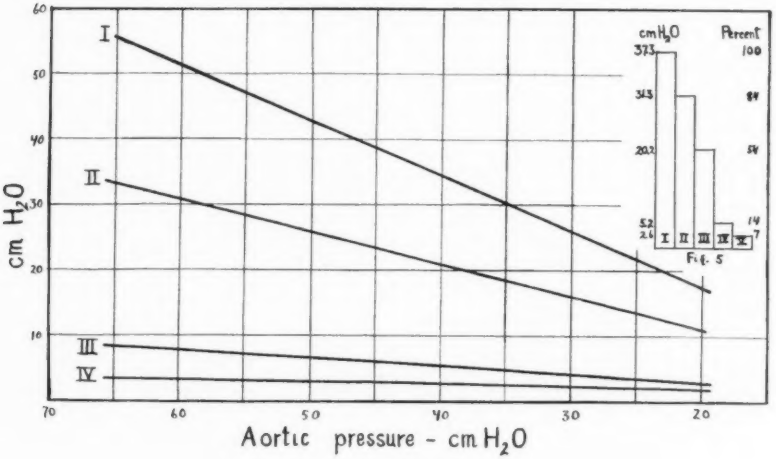



Fig. 3

Fig. 2. Camera lucida sketch of position of glomeruli supplied by different renal arteries in a small area of kidney. Solid dots represent glomeruli containing injection mass of one color; circles, those containing mass of different color.

Fig. 3. Tracing of photographs of mercury globules, showing successive changes in size and shape in passage from renal portal to renal vein: I, in renal portal vein; II, in branches of renal portal vein; III, in peritubular capillaries; IV, reassembled in branch of renal vein (×10).




Figs. 4 and 5

Fig. 4. Relation of average pressures in renal vessels to systolic aortic pressure: I, systolic afferent arteries; II, glomerular capillary; III, renal portal vein (peritubular capillaries); IV, renal vein.

Fig. 5. Diagram to show average pressure gradient in vessels of frog's kidney: I, systolic aortic; II, systolic afferent artery; III, glomerular capillary; IV, renal portal vein; V, renal vein.

from renal portal to renal vein. Small bubbles of air do pass through, but always in a tortuous and halting course. When a small globule of mercury is introduced into a cannula connected with the renal portal vein, its course through the kidney can be followed by observing first the dorsal and later the ventral surface. In the renal portal vein it is spherical, and passes readily into one of the branches of this vein toward the mesial part of the kidney. As the vein narrows, the mercury becomes elongated, then breaks into smaller globules, about 25 to 50 micra in diameter, which pursue tortuous courses through capillary sinusoids toward the ventral surface of the kidney. By now observing the ventral surface of the kidney, these small globules can be seen entering radicles of the renal vein, where they unite into larger globules again (fig. 3). The length of capillary, however, is short compared with the total length of vein through which the mercury passes, and may readily be overlooked if the injection is made hastily or under unnecessarily high pressure. Having been unable to confirm Smith's conclusions in many attempts, carried out as I conceive he did it, and having found that it is easy to deceive oneself if the injection is made other than very slowly and under conditions which permit examination of both aspects of the kidney, I cannot help but think he may have fallen into error in his interpretation of the experiments.

The intertubular vascular channels are relatively much larger in the frog than in the mammalian kidney. This has been commented upon by Hyrtl (1863), Vialleton (1902) and Shore (1901). From his studies of the development of the renal portal and posterior cardinal veins in the frog, Shore believes the intertubular channels are sinusoids rather than true capillaries.

*Blood pressure.* Estimation of the pressure in the afferent arteries and glomerular capillaries, with simultaneous estimations of aortic pressure, have already been presented (Hayman, 1927). To these are to be added estimations of the pressure in the renal portal and renal veins. The former are regarded as an indication of the pressure in the intertubular capillaries.

Venous pressures were measured by a water manometer, 2 mm. inside diameter, connected with a cannula in central end of renal portal or in the external iliac vein. The two methods yielded identical results. Another arm of the water manometer was connected with a 2 cc.-syringe filled with salt solution. Measurements were made by raising the column of salt solution in the manometer about a centimeter above venous pressure and observing the level to which it fell, then lowering the column by means of the syringe a few millimeters and observing the height to which the column rose. The two estimations agreed within 1 or 2 mm. in most instances. The manometric reading was corrected for difference in level of manometer and kidney and for capillarity. Pressures in the renal vein were estimated by inserting a cannula in the lowest branch of the renal vein. Simulta-

neous estimations of aortic pressure were obtained from a cannula in the left aortic arch connected with a Harvard manometer and were recorded on a smoked drum.

One hundred and one estimations of renal portal pressure and fifty-four of renal vein pressure were made on ten frogs under different conditions of the circulation. Estimations of pressure in the renal-portal-intertubular capillary system averaged 5.2 cm. $H_2O$ or 14 per cent of aortic pressure. The highest estimation was 9.2 cm. (aortic pressure 39 cm.), the lowest 2.5 cm. (aortic pressure 21 cm.). Of the 101 estimations 89 lay between 10 and 20 per cent of the aortic pressure simultaneously measured. The average of estimations of pressure in the renal vein was 2.6 cm. $H_2O$ or 7.1 per cent of aortic pressure. The average pressures in afferent artery, glomerular capillaries, intertubular capillaries (renal-portal) and renal vein at different aortic pressures is shown in figure 4. The representation of afferent arteriole and glomerular capillary pressures are taken from previous work (Hayman, 1927). The venous curves were drawn in the manner described at that time. The pressure gradient through the kidney vessels is diagrammed in figure 5 from the averages of all estimations. The marked fall in pressure between afferent glomerular artery and glomerular capillaries has been commented upon in an earlier paper. There is a similar fall between glomerular capillaries and intertubular spaces—the pressure in the former averaging 54 per cent of aortic pressure and in the latter only 14 per cent. No estimations of renal portal pressure exceeded the osmotic pressure of proteins of frog's plasma, as given by White (1924) and Landis (1928).

## CONCLUSIONS

Evidence is presented that in the kidney of *Rana pipiens:*

1. There is considerable overlapping in the distribution of adjacent renal arteries.
2. The number of arteriae rectae verae is very small.
3. The number of glomeruli in each kidney averages about 2000.
4. The efferent glomerular vessels and renal portal radicles unite in a common sinusoidal capillary plexus as described by Nussbaum.
5. Estimations of the pressure in the renal portal vein averaged 5.2 cm. $H_2O$ or 14 per cent of simultaneous estimations of aortic pressure.
6. Estimations of the pressure in the renal vein averaged 2.6 cm. $H_2O$ or 7.1 per cent of simultaneous estimations of aortic pressure.

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# NEURAL AND HORMONAL FACTORS IN BODILY ACTIVITY

## The Prepotency of Medulliadrenal Influence in Emotional Hyperglycemia

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Received for publication June 14, 1928

While certain independent reactions in isolated living tissues are well recognized it is no less patent that in the intact organism a rigidly controlled interdependence is *ab ovo* a characteristic of all activity. Undoubtedly there must arise from more intimate investigation into the integrative responses in the body many neural and hormonal relationships of far-reaching significance: to what degree bodily reactions may be said to be influenced by chemical as distinct from nervous intervention cannot, however, at present be answered. Since the demonstration about thirty years ago that specific substances which are elaborated by relatively minute organs in the body produce important and widespread effects on other diverse and distantly situated tissues, a gradually increasing emphasis has been placed on chemical or humoral influences on bodily activity.

Recently the dispensability of the sympathetic system from the superior cervical to the coccygeal ganglion inclusive has been demonstrated by Cannon, Lewis and Britton (1927); in the quiet conditions of the laboratory their animals passed apparently normal lives. Furthermore, the preponderating influence of humoral regulation over the neural in important central circulatory adjustments has been clearly pointed out in a report by Harrison, Blalock, Pilcher and Wilson (1927). While considerable ability was evident in the pansympathectomized animals which were observed throughout more than a year by Cannon and his associates, it should nevertheless be remarked that noteworthy functional defects appeared on exposure of such animals to necessitous conditions. And again, while the results of Harrison and his co-workers pointed towards significant chemical influences on the heart, the validity of other important and long-recognized neural effects on cardiac activity was not challenged.

Results which were derived from a recent series of experiments (Britton, Geiling and Calvery, 1928) demonstrated a very definite relationship of the adrenal medulla to carbohydrate metabolism. In a large group of

animals glycogen mobilization but not storage was profoundly affected in the absence of adrenin secretion, and the injection of minute amounts of

TABLE 1

*Emotional hyperglycemia and reactions in normal cats following brief periods of excitation (by dog)*

| CAT NUMBER | DATE | FASTING BLOOD SUGAR | TIME ANIMAL EXCITED | GENERAL REACTION | BLOOD SUGAR AFTER EXCITEMENT | | CHANGE IN BLOOD SUGAR |
|---|---|---|---|---|---|---|---|
| | | | | | 3–6 minutes | 15–20 minutes | |
| | | *mgm. per cent* | *minutes* | | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* |
| 1 | February 14 | 101 | 2 | + | 103 | 117 | +16 |
| 1 | February 23 | 83 | 2 | + | 93 | 96 | +13 |
| 2 | February 14 | 71 | 2 | + | 87 | 91 | +20 |
| 2 | February 23 | 74 | 2 | + | 86 | 83 | +12 |
| 3 | February 23 | 75 | 2 | +++ | 120 | 141 | +66 |
| 4 | February 23 | 158* | 2 | +++ | 233 | 228 | +75 |
| 4 | February 29 | 139* | 2 | +++ | 164 | 167 | +28 |
| 5 | February 29 | 80 | 2 | +++ | 119 | 125 | +45 |
| 6 | February 29 | 97 | 2 | +++ | 119 | 118 | +22 |
| 6 | March 6 | 103 | 2 | +++ | 125 | 135 | +32 |
| 7 | March 6 | 89 | 2 | +++ | 116 | 110 | +27 |
| 7 | March 10 | 98 | 2 | +++ | 127 | 115 | +29 |
| 7 | March 12 | 99 | 2 | +++ | 113 | 116 | +17 |
| 7 | March 15 | 103 | 4 | +++ | 160 | 151 | +57 |
| 7 | April 17 | 88 | 2 | + | 95 | 97 | +9 |
| 10 | March 8 | 84 | 2 | +++ | 117 | 151 | +67 |
| 10 | March 12 | 83 | 2 | +++ | 105 | 101 | +22 |
| 10 | March 15 | 77 | 4 | +++ | 100 | 99 | +23 |
| 10 | April 17 | 95 | 2 | + | 106 | 100 | +11 |
| 13 | April 18 | 76 | 4 | +++ | 111 | 97 | +35 |
| 13 | April 25 | 78 | 2 | + | 87 | 88 | +10 |
| 14 | April 5 | 78 | 4 | +++ | 118 | 129 | +51 |
| 14 | April 7 | 75 | 4 | +++ | 172 | 167 | +97 |
| 14 | April 17 | 153* | 2 | + | 171 | 187 | +34 |
| 15 | April 5 | 91 | 4 | +++ | 131 | 121 | +40 |
| 15 | April 17 | 85 | 2 | + | | 93 | +8 |
| 16 | April 5 | 78 | 4 | +++ | 95 | 105 | +27 |
| 16 | April 17 | 89 | 2 | + | 93 | 97 | +8 |
| 18 | April 25 | 82 | 2 | +++ | 113 | 109 | +31 |
| 18 | May 2 | 89 | 2 | + | 92 | 94 | +5 |
| 22 | May 29 | 73 | 2 | +++ | 96 | 86 | +23 |

* High blood sugars are probably referable to infection which appeared later in these cases.

insulin, although relatively innocuous before medulliadrenalectomy, brought about severe hypoglycemic convulsions from which spontaneous

recovery appeared impossible. Moreover it has been shown (Britton, 1928) that under certain circumstances normal hyperglycemic responses are absent in animals in which medulliadrenal secretion is suppressed. Further inquiry subsequently arose regarding the extent of the part played by the adrenal medulla, as well as and also distinct from that played by nervous influences, during a physiological hyperglycemic reaction. Because of its instinctive and fundamental nature as well as its typification of primal affective responses, the very common emotional response offered by a cat when confronted by a dog was utilized in the study.

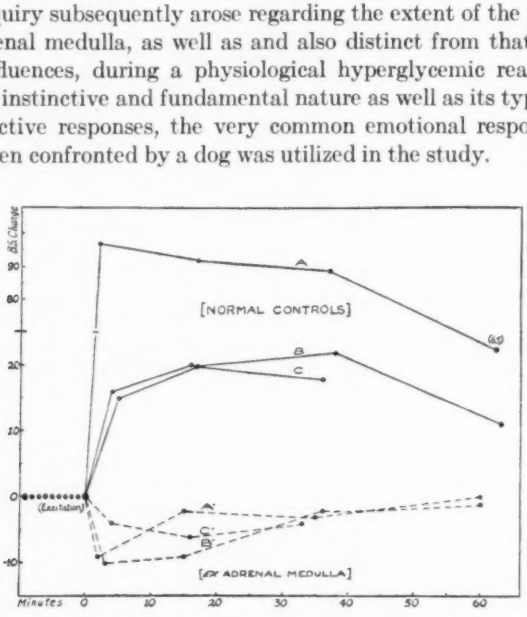


**Fig. 1. Curves depicting glycemic changes from the fasting levels in normal and in medulliadrenalectomized cats on emotional excitation. Zero minutes, time of withdrawal of dog from room. Continuous lines, normal controls; dash lines, adrenals ablated. Curves *A* and *A'*, cats 14 and 9, April 7th, excited by dog 4 minutes; general reactions were marked. *B* and *B'*, nos. 6 and 2, April 9th, excited 4 minutes; slight reactions. *C* and *C'*, cat 2, February 14th (when normal) and March 3rd (after medulliadrenal ablation), excited 2 minutes in each case; reactions slight.**

Cats which manifested a ready reaction to an aggressive dog were selected for the experiments. In the case of operated animals, in one group the adrenals were demedullated by making a small incision in the upper and in the lower pole of each gland and immediately applying curettage and thorough suction, by this means leaving practically all the highly important cortical tissue intact. In another group the liver was denervated by sectioning and stripping off for 1 to 2 cm. the sympathetic fibers (splanchnic branches) which course along the duodeno-hepatic artery. Controls were included with the three to six animals which were used at one time. After withdrawal from the ear vein of initial blood samples the

cats (previously fasted 12 hours) were caged and the dog then brought into the room. Dog A was used for cats 1 to 10 and dog B for the remainder of the animals; both were apparently of equal efficiency as exciting factors. Marked emotional reactions (designated +++ in tables) were those in which elevation of fur, hissing, snarling and energetic striking were observed, while slight reactions (+) were usually typified by disquietude resembling fear, crouching and only occasional thrusts at the aggressor. On completion of the excitatory period and removal of the dog further blood specimens were withdrawn, and the Folin-Wu (1920) method applied for making the sugar analyses. Glycogen determinations were made according to Pflüger's (1909) technique. A number of experiments in which changes in glycemia were observed in normal and operated animals when etherized to the excitement stage are reported in brief.

*Emotional hyperglycemia in normal animals.* Periods of excitation lasting only two minutes produced in normal animals considerable increments in blood-sugar percentage over the fasting levels (table 1). Maximal increases within 15 to 20 minutes after *initial* two-minute excitatory periods ranged from 15 to 75 mgm., while secondary and also later excitations for similar periods resulted in increases ranging from 5 to 34 mgm. per 100 cc.

Tables 1 and 4 give striking evidence of the diminishing glycogenolytic response of animals which were subjected repeatedly to emotional stimulation; in normal animals, however, in no case did the positive reaction disappear. Cats 7 and 10, which were observed on several occasions over periods of six weeks, continued throughout to show significant although diminishing blood-sugar increases. The fasting glycemia tended suggestively in many cases toward a higher level with successive emotional reactions.

More pronounced and prolonged hyperglycemias were elicited, it may be noted, following a period of four minutes' excitement: cat 14, for example, showed on April 7 an appreciation in blood sugar of considerably over 100 per cent, and furthermore a high glycemic level was sustained for an hour after withdrawal of the dog from the room. These facts are reproduced graphically in figure 1.

*Hepatic (sympathetic) denervation and hyperglycemic reactions.* Complete removal of the nerve fibers which transmit glycogenolytic impulses to the liver, without interference to medulliadrenal function, resulted usually in somewhat modified glycemic responses to emotional excitation. The observed general reactions were, nevertheless, often extremely vigorous.

Animal 1 should perhaps not be regarded as typical in this group (table 2): it had been exposed in two experiments before hepatic sympathectomy to a barking, attacking dog, and also many of its affective reactions were of slight degree only. If the more vigorous reactions are considered, the

(corresponding) glycemic changes are quite significant although smaller than those evoked from normal animals. It is conceded as possible though improbable that partial regeneration of the hepatic nerves had occurred at the time of the later experiments. Slightly subnormal glycogenolytic responses were also characteristic of no. 11, but in every instance the changes were of positive sign.

TABLE 2

*Hyperglycemic responses of animals following emotional excitation. Hepatic nerves severed in all cases: adrenal medulla active*

| CAT NUMBER | DATE HEPATIC NERVES CUT | DATE OF EXPERIMENT | FASTING BLOOD SUGAR | TIME ANIMAL EXCITED | GENERAL REACTION | BLOOD SUGAR AFTER EXCITEMENT | | CHANGE IN BLOOD SUGAR |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 3–6 minutes | 15–20 minutes | |
| | | | *mgm. per cent* | *minutes* | | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* |
| 1 | February 27 | March 3 | 80 | 2 | + | 80 | 85 | +5 |
| 1 | February 27 | March 6 | 94 | 2 | +++ | 102 | 96 | +8 |
| 1 | February 27 | March 8 | 78 | 2 | + | 76 | 72 | −6 |
| 1 | February 27 | March 12 | 77 | 2 | + | 69 | 68 | −9 |
| 1 | February 27 | March 15 | 84 | 4 | +++ | 82 | 91 | +7 |
| 1 | February 27 | March 17 | 96 | 4 | + | 89 | 94 | −7 |
| 1 | February 27 | April 16 | 96 | 2 | +++ | 101 | 104 | +8 |
| 1 | February 27 | May 2 | 95 | 2 | + | 102 | 105 | +10 |
| 1 | February 27 | May 11 | 97 | 2 | +++ | 93 | 104 | +7 |
| 8 | March 14 | March 17 | 89 | 4 | +++ | 106 | 115 | +26 |
| 8 | March 14 | April 4 | 88 | 4 | +++ | 130 | 113 | +42 |
| 8 | March 14 | April 7 | 85 | 2 | + | 89 | 92 | +7 |
| 8 | March 14 | April 16 | 101 | 2 | + | 112 | 109 | +11 |
| 11 | March 14 | March 17 | 120 | 4 | +++ | 133 | 133 | +13 |
| 11 | March 14 | April 4 | 100 | 4 | +++ | 104 | 107 | +7 |
| 11 | March 14 | April 7 | 90 | 2 | +++ | 97 | 93 | +7 |
| 11 | March 14 | April 16 | 97 | 2 | +++ | 112 | 104 | +15 |

In cat 8, however, very definite and considerable glycogen-mobilizing ability, even in the complete absence of splanchnic fibers reaching to the liver, was evident. Within a week following hepatic denervation this animal showed an augmentation of blood sugar on emotional excitation of over 25 per cent, while three weeks after operation an increase of almost 50 per cent above the fasting level was observed. The increases in these experiments approximate, therefore, and in some instances more than equal, those derived from normal controls.

Elimination of the adrenal medulla in the case of cat 8 brought about a

TABLE 3

*Glycemic changes and reactions produced by emotional excitement of animals with adrenal medulla extirpated and hepatic nerves intact*

| CAT NUMBER | DATE ADRENAL MEDULLA EXTIRPATED | DATE OF EXPERIMENT | FASTING BLOOD SUGAR | TIME ANIMAL EXCITED | GENERAL REACTION | BLOOD SUGAR AFTER EXCITEMENT | | MAXIMAL CHANGES IN BLOOD SUGAR |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 3-6 minutes | 15-20 minutes | |
| | | | *mgm. per cent* | *minutes* | | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* |
| 2 | February 27 | March 3 | 105 | 2 | + | 101 | 99 | −6 |
| 2 | February 27 | March 6 | 96 | 2 | +++ | 89 | 83 | −13 |
| 2 | February 27 | March 8 | 102 | 2 | + | 92 | 87 | −15 |
| 2 | February 27 | March 12 | 91 | 2 | + | 88 | 90 | −3 |
| 2 | February 27 | March 15 | 84 | 4 | +++ | 74 | 74 | −10 |
| 2 | February 27 | March 17 | 85 | 4 | +++ | 85 | 85 | 0 |
| 2 | February 27 | April 9 | 85 | 4 | + | 75 | 76 | −10 |
| 2 | February 27 | April 16 | 92 | 2 | + | 90 | 84 | −8 |
| 2 | February 27 | May 2 | 93 | 2 | +++ | 86 | 80 | −13 |
| 2 | February 27 | May 11 | 91 | 2 | + | 85 | 88 | −6 |
| 9 | March 7 | March 10 | 96 | 2 | +++ | 91 | 83 | −13 |
| 9 | March 7 | March 13 | 84 | 2 | +++ | 81 | 75 | −9 |
| 9 | March 7 | March 17 | 98 | 4 | +++ | 98 | 95 | −3 |
| 9 | March 7 | April 4 | 89 | 4 | +++ | 92 | 85 | −4 |
| 9 | March 7 | April 7 | 87 | 4 | +++ | 78 | 85 | −9 |
| 12 | March 14 | March 17 | 135 | 4 | +++ | 134 | 132 | −3 |
| 12 | March 14 | April 4 | 93 | 4 | +++ | 94 | 91 | −2 |
| 12 | March 14 | April 16 | 110 | 2 | + | 105 | 107 | −5 |
| 12 | March 14 | April 18 | 96 | 4 | + | 90 | 79 | −17 |
| 12 | March 14 | April 25 | 89 | 2 | + | 88 | 89 | −1 |
| 12 | March 14 | May 2 | 89 | 2 | +++ | 88 | 84 | −5 |
| 12 | March 14 | May 11 | 108 | 2 | +++ | 101 | 102 | −7 |
| 17 | April 19 | April 25 | 117 | 2 | +++ | 111 | 97 | −20 |
| 17 | April 19 | May 2 | 87 | 2 | +++ | 87 | 85 | −2 |
| 19 | May 17 | May 23 | 92 | 2 | +++ | 95 | 84 | −8 |
| 19 | May 17 | May 29 | 84 | 2 | +++ | 80 | 74 | −10 |
| 20 | May 19 | May 23 | 97 | 2 | +++ | 83 | 88 | −14 |
| 20 | May 19 | May 29 | 113 | 2 | +++ | 104 | 108 | −9 |
| 21 | May 19 | May 23 | 99 | 2 | +++ | 98 | 95 | −4 |
| 21 | May 19 | May 29 | 84 | 2 | +++ | 78 | 81 | −6 |

marked difference, however, in the glycogenolytic response on excitation. Whereas substantial increments in blood sugar had characterized the reactions of this animal for some weeks after hepatic denervation, decrements only were observed subsequent to adrenal medullectomy (performed on April 19). Six days after operation the fall on excitation was from 98 mgm. to 94 mgm. per cent, and one week later a diminution of 13 mgm. from the fasting blood-sugar level was recorded.

*Emotional reactions in animals deprived of medulliadrenal tissues.* In distinct contrast to the control cases are the results which were obtained

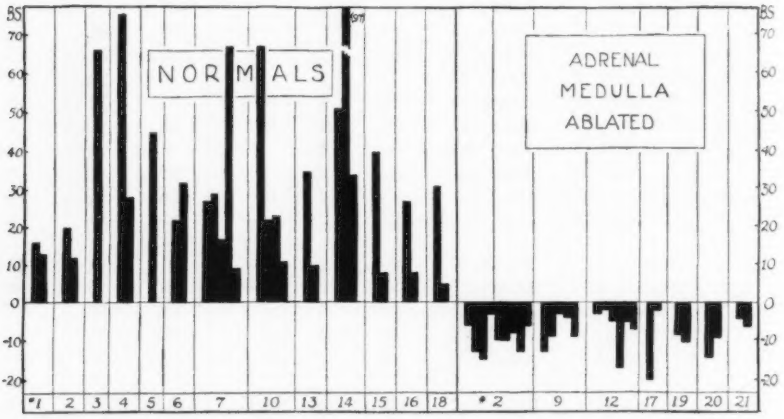


Fig. 2. Chart indicating hyperglycemic reactions in normal animals, and decrements in blood sugar in animals without adrenal medulla, following emotional excitement. Ordinates, changes in blood sugar from fasting (0) level; abscissae, animal numbers. All cases are listed. At a glance the striking difference in reaction may be appreciated.

consistently with medulliadrenalectomized animals. Reference to the last column in table 3 discloses the striking fact that in no instance was the maximal change in blood-sugar level following emotional excitation a positive one; in practically all cases, indeed, depressions were observed.

In one-third of the experiments the glycemic percentage fell between 10 and 20 mgm. While cat 2 had displayed normal hyperglycemic responses after two-minute periods of excitement previous to operation (see table 1), considerable decrease followed two or even four-minute periods of excitation when given some weeks after removal of the adrenal medulla (table 3).

Moreover, six demedullated animals which had not previously been exposed in the laboratory to an active, barking dog failed to maintain the

fasting blood-sugar level on emotional stimulation (see table 3), although in a majority of cases the apparent affective reactions were extremely pronounced. Even after four minutes' excitation no definitely positive results were forthcoming. Two of the animals, cat 9 on April 4th and cat 20 on May 23rd, showed insignificant increments within 3 to 6 minutes after stimulation, followed shortly afterwards in each case by a more noteworthy decrease.

Comparison is made in figure 1 of the results in some of the foregoing experiments with those derived from normal animals. Glycemic changes on emotional stimulation in the two main groups of animals are summarized graphically in figure 2. Very striking disabilities are present, it readily appears, in those animals lacking normal adrenin secretion.

In three animals medulliadrenalectomy as well as hepatic denervation was carried out in one operation, and in these cases also depressions in blood sugar followed various periods of emotional excitement.

On one normal control animal, cat 6, a dummy operation simulating adrenalectomy without damage to the adrenal medulla was performed on March 7. Only three days later the response on exposure to the dog was definitely positive—the blood sugar rose from 79 mgm. to 97 mgm. per cent five minutes after excitation. Within a few weeks after operation the emotional hyperglycemic reaction was more pronounced.

*Hyperglycemia following excitement from ether administration.* In several normal animals the changes in blood sugar incident to exposure to ether (20 cc. sprayed in a bell-jar containing 20 liters air) were observed, and comparison was made with those changes found in animals in which the glycogenolytic factors had been reduced to a minimal degree by hepatic denervation and medulliadrenal removal.

The increments in normal animals on exposure to ether until reaching the excitement stage were of extreme degree. In one animal, cat 1, on February 25th, the increase in glycemia was from 76 mgm. (fasting level) to 182 mgm. per cent within 20 minutes after a 5-minute period of ether administration. Another animal, no. 2, similarly treated, showed a rise from 79 mgm. (normal) to 179 mgm. within 5 minutes after removing the anesthetic. Cat 3, exposed to ether on the same date for 3 minutes increased its blood sugar from 81 mgm. to 110 mgm.

In the absence of the important nervous and humoral factors which directly influence glycogen mobilization, however, ether administration still effected notable but not so considerable changes in blood-sugar concentration. Cats A and B were operated on on January 17th and January 24th respectively, the adrenals being demedullated and the hepatic nerves sectioned in each case. On February 25th cat A showed an increase from 77 mgm. to 128 mgm. within 20 minutes after a 5-minute exposure to ether

vapor, and cat B from 100 mgm. to 111 mgm. after 3 minutes under the anesthesia bell-jar.

The well-known toxic effects of ether (see Rosenthal and Bourne, 1928, also Long, 1928), particularly on hepatic tissues, admittedly complicate these experiments. It is clear, however, that under exceedingly severe (traumatic) stimulation glycogen may be released from the storage depots in medulliadrenalectomized, hepato-denervate preparations. Glycemic changes in such animals were nevertheless approximately only half as great as those found in normal controls.

*Glycogen content of liver in operated animals; necropsy.* Several animals were guillotined at the end of the experiments and determinations of hepatic glycogen immediately carried out. Cat 1, in which the hepatic nerves were severed on February 27th, showed 3.40 per cent liver glycogen; nos. 2 and 12, medulliadrenalectomized on February 27th and March 14th respectively, 4.00 per cent and 1.53 per cent; and no. 8, in which the hepatic nerves were cut on March 14th and the adrenal medulla evacuated on April 19th, 3.53 per cent. These determinations were made on May 11th, the animals being killed within a few minutes, it should be noted, after marked emotional excitation of the medulliadrenalectomized animals had failed to evoke a hyperglycemic response—had resulted, indeed, in noteworthy depressions in blood sugar.

In accord with the foregoing findings are those previously published from this laboratory (Britton, Geiling and Calvery, 1927) and also those reported by other workers (see Stewart and Rogoff, 1918).

At necropsy the (demedullated) adrenals of those animals which are grouped in table 3 appeared as approximately half-sized glands with normal vascular relations, and on macroscopic dissection gave evidence of containing adrenal cortical tissue only. In the hepato-denervate preparations thick connective tissue growths about the duodeno-hepatic artery precluded definite naked-eye determination regarding the regeneration of nerve fibers. Since most of the experiments were performed within a few weeks after operation, however, the possibility of functional regenerated nerves can safely be outruled.

Discussion. Glycosuric conditions occurring under various forms of excitatory stimulation have been frequently described. The "Fesselungs-diabetes" which was observed by Böehm and Hoffmann (1878) in animals bound to a holder for varying periods was probably largely referable to cooling effects and pain incident to operative procedures. Later Cannon, Shohl and Wright (1911) demonstrated that glycosuria was readily induced in normal cats which were faced by an active dog while placed under restraint, but that the phenomenon was quite absent in animals which had been bilaterally adrenalectomized. Considerably increased amounts of circulating adrenin have been found (Cannon and de la Paz, 1911; Cannon

and Britton, 1927) in the blood of emotionally excited animals. Under conditions of severe muscular exertion and of emotional stress in man the presence of urinary sugar has also been frequently noted (Folin, Denis and Smillie, 1914; Malmiwirta and Mikkonen, 1924; Tigerstedt, 1926). Folin interestingly states that 18 per cent of the medical students observed after an important examination and several insane hospital patients showed unmistakable glycosuria.

Acapnia was advanced at one time by Henderson and Underhill (1911) as being usually responsible for traumatic as well as emotional glycosurias. Stewart and Rogoff, too, have on many occasions (1918–23–24) brought forward negative evidence regarding adrenal effects on carbohydrate metabolism. More recent investigations of a definitely positive nature which have been forthcoming (Cannon, McIver and Bliss, 1924; Britton, Geiling and Calvery, 1928) do not appear, however, to leave room for reasonable doubt of adrenal involvement, particularly of the medulla, in the regulation of normal sugar metabolism in the body.

Hitherto the influence of medulliadrenal secretion and of splanchnic impulses to the liver in a physiologically-produced hyperglycemic reaction does not appear to have been differentiated. Some related observations made in 1909 by Gautrelet and Thomas and in 1911–12 by Macleod and his associates suggested, nevertheless, an adreno-splanchnic relationship in vasomotor and glycogenolytic reactions. In the pseudaffective (decorticate) preparation Bulatao and Cannon (1925) have also demonstrated very clearly that the adrenal glands are extremely important in the development of hyperglycemia during the quasi-emotional state. The present report contributes experimental evidence which points very definitely to prepotency of the adrenal medulla in producing the hyperglycemia incident to profound emotional disturbances.

In a retrospective paper Schafer (1927) has remarked on the probability that neural influences may play the primary part in inhibiting and controlling animal reactions, and that the part played by humoral influences, although in many cases a useful and even a necessary auxiliary, is nevertheless secondary to that played by the nervous system. He also draws the conclusion that functions which are controlled both neurally and humorally are initiated by direct nervous influence and continued by the humoral influence, the object of the latter being to prolong the effect of the former and thus to bring about an economy of nervous energy.

Reference to the summary of results of the foregoing experiments as presented in table 4, however, discloses the very marked inability of animals which are deprived of a humoral factor such as adrenin to respond by releasing glycogen reserves under the demands of an extreme physiological contingency. In practically all the medulliadrenalectomized cases the normal fasting glycemic level was not even maintained on subjection of

the animals to strong emotional excitation—indeed 10 to 25 per cent decrements were registered in many instances. In animals with the adrenal glands intact, which were similarly excited, it may be noted that the blood-sugar increases ranged from 30 to over 100 per cent. Ablation of the neural glycogenolytic pathways by section of the splanchnic hepatic branches, moreover, although possibly modifying the response in some cases, did not, in many of the experiments, appreciably depress the discharge of glucose from the liver.

These findings do not appear to lend support, therefore, to the probability which Schafer advances, since medulliadrenal influence in the liberation of glycogen stores under conditions of urgency is undoubtedly of pre-eminent

TABLE 4

*Summary of blood sugar changes following emotional reactions in animals under normal and other experimental conditions*

In each case the maximal variations in blood sugar from the fasting level are given, in mgm. per cent, as observed within 20 minutes after excitation of cat.

| ANIMALS NORMAL | | | HEPATIC NERVES SEVERED | | ADRENAL MEDULLA ABLATED | |
|---|---|---|---|---|---|---|
| Excited 2 minutes | | Excited 4 minutes | Excited 2 minutes | Excited 4 minutes | Excited 2 minutes | Excited 4 minutes |
| First excitations | Later excitations | | | | | |
| +16 | +13 +22 | +57 | +5 | +7 | −6 −5 | −10 |
| +20 | +12 +11 | +23 | +8 | −7 | −13 −7 | 0 |
| +66 | +28 +10 | +35 | −6 | +26 | −15 −20 | −10 |
| +75 | +32 +34 | +51 | −9 | +42 | −3 −2 | −3 |
| +45 | +29 +8 | +97 | +8 | +13 | −8 −8 | −4 |
| +22 | +17 +8 | +40 | +10 | +7 | −13 −9 | −9 |
| +27 | +9 +5 | +27 | +7 | | −6 −14 | −3 |
| +67 | | | +7 | | −13 −10 | −2 |
| +31 | | | +11 | | −9 −4 | −17 |
| +23 | | | +7 | | −5 −6 | |
| | | | +15 | | −1 | |

importance. A relatively minor rôle, indeed, in the absence of normal secretion from the adrenal medulla, is subserved by the hepato-sympathetic fibers. Rather than the humoral acting merely as a supporting and secondary influence to the neural, therefore, the reverse may even hold true. The organism lacking humoral fortification appears under stressful conditions to be distinctly defective glycogenolytically.

Larger increments in blood sugar in normal animals were observed to follow the longer periods of excitation (see table 4). Furthermore, a gradually increasing tolerance towards the excitation was evidenced: the average increase in glycemia which occurred in 14 secondary or later

exposures to the dog scarcely approximated one-half the average increase observed in primary exposures.

In a small animal such as the cat, weighing between 3 and 4 kilos, it may be derived from the foregoing experiments that a total of 50 to 250 mgm. of glucose may within a few minutes be released from the liver into the blood stream. This occurs evidently in anticipation of the demand, and through actuation chiefly by the adrenal medulla. In conditions endangering the life of an animal the exceeding importance of such a ready augmentation of the circulating nutrient material for use by the active (particularly muscular) tissues is apparent.

## SUMMARY

Normal cats which have been excited (while caged, and displaying only minor muscular activity) by an aggressive dog show marked increments in blood-sugar level. Following two minutes' excitement the glycemic percentage is commonly augmented 30 to 90 per cent; greater increases (up to over 100 per cent) are observed after four minutes' excitation. The hyperglycemic as well as the general reactions become less marked with succeeding emotive responses. Evidences of parallelism in visceral and somatic responses are apparent.

Hyperglycemia attending emotional reactions in animals with splanchnic branches to the liver severed and normal medulliadrenal function maintained is only slightly modified from the normal.

Profound disability of the glycogenolytic mechanism is observed in animals which have been deprived of the adrenal medulla; emotional excitation for two or four minutes in such cases usually results in significant depressions in blood sugar which may persist for twenty minutes or longer. The general affective response is not appreciably altered.

The hepatic glycogen in medulliadrenalectomized animals was within the normal limits. The observed emotional hypoglycemia, therefore, was probably referable to failure to mobilize glycogen, in the absence of adrenin secretion, adequately to supply the demands of even minor muscular activity.

The importance of medulliadrenal intervention in calling forth readily available fuel for muscular activities during conditions of urgency is pointed out.

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# THE HORMONE OF THE ADRENAL CORTEX

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Received for publication June 23, 1928

After many attempts to obtain the hormone of the adrenal cortex we are now ready to report positive results. We used upwards of one hundred cats and many different adrenal preparations in the preliminary investigations which preceded the results published in this communication. Only a brief report of some of these experiments has been published (1).

We concluded from this earlier work that some method should be found which would remove the epinephrin without destroying the cortical hormone because the presence of epinephrin in appreciable amounts had proven detrimental.

At the present time the only absolute test of the potency of cortical extracts is their efficacy in prolonging the life of completely adrenalectomized animals.

Cats have been used exclusively in our work.

Needless to say, one cannot make a satisfactory use of such animals without considerable experience. The operative technique and the care of animals afterwards must be uniform.

*Animal technique.* In all of the experiments reported in this paper, the adrenals have been removed through the lumbar path in two stages by the same operator and experienced assistants. The interval between operations has usually been from two to seven days. Aseptic technique has been carefully followed.

The animals have not been fed until forty-eight hours after the final operation because of the ill-effects sometimes resulting from earlier feeding. A record was kept of the amount of food eaten each day. Canned salmon with occasional meals of beef served as the diet.

The temperature of the hospital was maintained at 27° to 28°C. by a thermostat. Practically all injections were subcutaneous. At first they were made every four to six hours throughout the life of the animal, later only twice daily.

In animals which survived a week or more, careful post-mortem search was made for the presence of accessory cortical bodies.

*Preparation of extracts.* Fresh beef adrenals chilled soon after removal

from the animals, were transported to the laboratory where the cortex was carefully separated from the medulla. After being ground either in a mortar or meat chopper the cortex was shaken with 3 volumes of water for fifteen to thirty minutes. Acetic (0.1 N) acid was then added in just sufficient amount to produce ready filtration. Solids were separated in the earlier work by gravity filtration and later by centrifugalization. This clarified liquid served as the starting point in all preparations described in this paper, unless otherwise stated, and will be designated as the acetic extract. The pH ranged from 4.4 to 4.9. The 1:3 cortical extract before the addition of acid was about pH 6.6 (determined electrolytically by Dr. Geo. Pucher). If used for injection the extract was neutralized. One cubic centimeter contained about 0.2 gram of the cortex.

The next step had as its primary object the removal of epinephrin. The presence of epinephrin was determined by the method of Folin, Cannon and Denis (2).

I. $KMnO_4$ in dilute solution was added drop by drop to the acetic extract until practically all of the epinephrin was destroyed.

II. Weighed quantities of blood charcoal were shaken with the acetic extract until most of the epinephrin was removed. The mixture was then filtered.

III. The acetic extract was *dialyzed* against a large volume of isotonic NaCl through a collodion membrane for 12 to 18 hours.

IV. On the possibility that the hormone could pass more easily through a thin membrane than through a thick one, extract for injection was prepared by placing a measured quantity of acetic extract in a thin collodion sac which in turn was immersed in an equal volume of isotonic salt solution contained in a thick collodion sac of about double the capacity. The combined sacs were then immersed in a large volume of isotonic salt solution. Dialysis was carried out in the refrigerator for 18 hours. The solution outside of the thin sac but inside of the thick one was used for injection.

HCl was employed in another series of preparations.

V. *a.* Cortical pulp shaken with twice its volume of water was filtered through glass wool. The filtrate was dialyzed for 19 hours in the cold against a large volume of water to remove epinephrin. One and four-tenths cubic centimeters of *N*HCl was then added to every 100 cc. of the residue and the material again dialyzed to remove more epinephrin. The final residue was neutralized and used for injection.

*b.* Cortical pulp was extracted with thrice its volume of water, then 9 cc. of *N*HCl were added for every 100 cc. of extract. This was then dialyzed against a large volume of acidulated water for several hours to remove epinephrin. The residue was neutralized and used for injection.

*c.* As above, except the epinephrin was removed by charcoal.

*d.* The ordinary acetic extract was treated with 5 cc. of *N*HCl per 400 cc. of extract and allowed to stand for 30 minutes before neutralizing. Dialysis was carried out against an equal volume of 0.9 per cent NaCl solution through thin collodion.

We next attempted to remove the active substance by precipitation.

VI. Enough trichloracetic acid was added to the acetic extract to form a good precipitate. The precipitate was washed then redissolved in water by the addition of just enough NaOH to carry it into solution. A sufficient amount of NaCl was added to make it isotonic with the body fluids.

VII. *a.* The acetic extract was saturated with NaCl. The precipitate was washed with saturated NaCl solution to remove the epinephrin. Filtration was carried out by gravity. The precipitate was redissolved in water so that 1 cc. contained the material from 1 gram of cortex.

*b.* In a second series the NaCl content of the final extract was made 0.9 per cent and the pH was adjusted to 7.0.

*c.* Extract prepared according to *b* was kept in the refrigerator for *thirty days* before use.

*d.* The NaCl precipitate was redissolved in only one-half of the usual quantity of water and then dialyzed through a thin collodion membrane against 0.9 per cent NaCl solution on a shaking machine for four hours at room temperature. If the membrane were completely permeable and equilibrium had been reached in the time allowed 1 cc. of the dialysate should contain the material from 1 gram of cortex.

*e. Third precipitate.*

Purification was attempted by reprecipitation. Each time except the first the pH of the extract was adjusted to 5.4 preparatory to saturation with NaCl. Each time the precipitate was redissolved the pH was adjusted to 7.0. Filtration was by gravity. The precipitate became less and less soluble with each precipitation.

VIII. *a. Precipitate stage short.*

After finding that much of the potency was lost when the material was allowed to stand in the form of a precipitate, a high speed centrifuge was used to shorten this stage. Otherwise the material was prepared as already described under VII *b* except that the pH was 7.35.

*b.* Second precipitate. Extract of the first precipitate was reprecipitated. One cubic centimeter of the second precipitate extract was equivalent to 5 grams of cortex.

All extracts were sterilized by filtration through a Seitz filter. Usually they were not kept longer than 5 days before use.

RESULTS. *Controls.* As controls we have used cats injected with Ringer's solution and cats not injected. Many of the controls were run at the same time as those treated with cortical material so as to be under the influence of the same conditions.

Large amounts of Ringer's solution, 11 to 20 cc. per kilo per 24 hours, prolong the life of adrenalectomized cats. Ten animals so treated by us survived between five and sixteen days, averaging nine days. This is a shorter period than that reported by Marine and Baumann in cats similarly treated.

Quantities of Ringer's solution up to 5 cc. per kilo per 24 hours do not prolong the life of adrenalectomized cats. Nine animals so treated have lived an average of 5.7 days. This period agrees with other results in this laboratory where insufficient amounts of cortical extracts were given. Marine and Baumann (3) give 5.3 days as the average survival period for adrenalectomized untreated animals.

*Comparison of different extracts.* Table 1 shows the effect of acetic extracts on the survival of adrenalectomized cats both with and without the removal of epinephrin.

TABLE 1

*Efficacy of acetic extracts*

| EXTRACT | TOTAL CC. IN 24 HOURS PER KILO | NUMBER OF CATS USED | RANGE OF SURVIVAL DAYS | AVERAGE SURVIVAL DAYS |
|---|---|---|---|---|
| Unmodified | 12–17 | 4 | 2–9 | 4.4 |
| I. Epinephrin destroyed by $KMnO_4$ | 20 | 2 | 7–9 | 8 |
| II. Epinephrin removed by charcoal | 10–31 | 9 | 6–36 | 12.2 |
| III. Epinephrin removed by dialysis, residue injected | 11–22 | 8 | 3–38 | 11.7 |
| Same | 3–4.6 | 5 | 2–22 | 12.0 |
| IV. Dialysate | 3–4.6 | 6 | 2–27 | 13.8 |

It will be noted that extracts from which the epinephrin was removed by charcoal and by dialysis were about equally potent.

Compared with an average survival of 5 to 6 days for untreated animals 12 days indicated that the hormone was present in the extract.

The shortened survival period for cats treated with unmodified acetic extract could be accounted for by the epinephrin content.

Treatment of cortical material with HCl did not yield better results (table 2). We seemed to be making no progress with our methods of separation until we tried to precipitate the hormone.

Table 3 shows the effects of extracts made from precipitates of the acetic acid preparation. The trichloracetic preparation was valueless judging from the very limited number of test animals. The animal marked 7 lived 36 days but not from the injection of this preparation. At the end of seven days he showed distinct signs of adrenal insufficiency particularly weakness in the hind legs. "Charcoal acetic" extract (II) was given, whereupon he gradually improved until apparently normal.

The importance of controlling the pH and also the NaCl content is shown in VII*b* in comparison with VII*a*. It must be admitted, however, that the latter series is small and respiratory infections played a part in reducing the period of survival in three animals.

There seems to be a loss in potency if the extract is kept several days before use (VII*c*).

The active substance appears to be dialyzable (VII*d*).

The first attempts to purify by reprecipitation were not very successful (VII*e*). This might have been due to much active substance becoming insoluble if it remained in the precipitated form long. Evidence for this had been noted in the apparent loss of potency of some first precipitate extracts which because of unusually slow filtration necessitated a marked prolongation of the precipitated stage.

On the strength of this supposition a new method was tried. The use of a super-centrifuge reduced the time required to separate and wash the

TABLE 2

*Efficacy of HCl extracts*

| EXTRACT | TOTAL CC. IN 24 HOURS PER KILO | INDIVIDUAL SURVIVAL DAYS | AVERAGE SURVIVAL DAYS |
|---|---|---|---|
| V. a. Dilute HCl | 3–5 | 5, 8, 18 | 10 3 |
| V. b. Stronger HCl | 1–4 | 8, 9, 9, 9, 12, 12, 19.5, 20 | 12 3 |
| V. c | 3 | 6.5, 8 | 7 2 |
| V. d. Residue | 3 | 7 | |
| Dialysate | 3 | 9 | |

precipitate from a few hours to about half an hour. Results obtained by this method are shown in table 4.

The average survival of first precipitate treated cats was but a little greater than that for cats treated by the old method (VII*b*). The cat which survived 80 days died because the extract was discontinued. This animal was doing so well at 69 days that injections were stopped. On the eightieth day the animal was found prostrated. Attempts to improve the condition by the injection of extract were futile. No accessory tissue was found. The cat that survived 31 days was found with the whole ramification of small bronchioles plugged with pus. Three other cats had their survival periods shortened by infections, two respiratory and the other cutaneous.

With this last method there does not appear to be the loss in potency from reprecipitation which was found in the previous method. Indeed there appeared to be improvement from reprecipitation. Although only

four animals have been treated (VIII*b*) they show a higher average than that for any previous extract.

*Response of adrenalectomized animals to cortical extract.* Besides the period of survival in adrenalectomized animals one obtains some indication of the efficacy of an extract by the behavior of the animal. This will be treated more at length in the paper which follows. However, we wish to point out here that the administration of extracts to animals showing an advanced stage of asthenia fails to produce recovery. Occasionally there

TABLE 3

*Efficacy of precipitate extracts*

| PREPARATION | TOTAL CC. IN 24 HOURS PER KILO | NUMBER OF CATS USED | INDIVIDUAL SURVIVAL DAYS | AVERAGE SURVIVAL DAYS |
|---|---|---|---|---|
| VI. Trichloracetic | 10–18 | 3 | 2, 4.7, 7 | |
| VII. a. NaCl precipitate | 2.7–5.4 | 5 | 8, 17, 17, 18, 18 | 15.6 |
| VII. b. Same, but pH and NaCl content controlled | 2–4 | 12 | 11, 11, 14, 21, 22, 22.5, 24, 27, 28, 31, 40, 60 | 25.6 |
| VII. c. Same, kept 30 days | 2.4–3.6 | 6 | 3.7, 6.5, 7.3, 8.5, 26, 40 | 15.3 |
| VII. d. Dialysate | 2–4 | 9 | 10, 13, 14.5, 15, 15, 23, 24, 26, 31 | 19 |
| VII. e. Third precipitate | 3–4 | 5 | 3.5, 8.5, 8.5, 9.5, 13 | 8.6 |

TABLE 4

*Results from NaCl precipitates separated and washed by the super-centrifuge*

| PREPARATION | TOTAL CC. PER KILO IN 24 HOURS | INDIVIDUAL SURVIVAL DAYS | AVERAGE SURVIVAL DAYS |
|---|---|---|---|
| VIII. a. First precipitate | 3–4 | 11.5, 12, 13.5, 17, 20, 22, 31, 33.5, 34, 80 | 27.4 |
| VIII. b. Second precipitate | 2–3 | 24, 28, 31, 41.5 | 31.1 |

may be temporary improvement but it is of little consequence. On the other hand cats which are beginning to show the first evidences of asthenia, a slight unsteadiness in the hind legs, may recover under treatment.

If the animal has reached the stage of prostration, extract is practically useless.

DISCUSSION. We have furnished sufficient evidence that the adrenal cortex produces a hormone. It can be salted out with NaCl.

The name *Cortin* has been proposed for this hormone, in preference to corticin because the latter is the name of an alkaloid obtained from the bark of *Populus tremula*.

Rogoff and Stewart have recently reported extracts of the adrenal which prolong the lives of adrenalectomized dogs. However they do not describe their method of preparing the extracts (4).

SUMMARY

The hormone of the adrenal cortex has been salted out with NaCl.

*Cortin* is proposed as the name for this substance.

Completely adrenalectomized cats injected twice daily with this substance have survived an average of 27.4 days or longer as compared with 5 to 6 days for controls.

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# OBSERVATIONS UPON ADRENALECTOMIZED CATS TREATED WITH THE CORTICAL HORMONE

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Received for publication June 23, 1928

In the preceding paper we have described a method of separating the hormone from the adrenal cortex. Adrenalectomized cats were used as test objects. This paper is an account of the observations made upon those animals.

The prolongation of the period of survival after complete adrenalectomy, by the use of extracts, has made possible a study of what most nearly approaches chronic adrenal insufficiency of any experimental condition thus far produced in cats.

One hundred and eight animals served as material for this work.

We do not propose to treat of all symptoms observed because this would be but a repetition of many excellent descriptions of adrenal insufficiency found in the literature of which Elliott's (1) is one of the best.

METHODS. The operative technique and care of the animals is described in the preceding communication.

Extracts were injected subcutaneously twice in twenty-four hours. Not more than 10 cc. was injected per day. This contained material from about 10 grams of cortex.

Blood ureas were frequently determined by the usual urease aeration method on blood taken from the ear. Metabolism was determined in a few animals. For this the Haldane method was used in all except two animals. Details concerning this method are given in connection with the results.

*Response of adrenalectomized cats to extracts.* Injections were begun immediately after the removal of the second adrenal and continued with few exceptions for the remainder of the animal's life. Usually the animal would recover from the operation almost as well as though the adrenals were intact. He might then go on with little variation until shortly before death when the appetite would gradually disappear and he would lose interest in his surroundings. Again the animal might show fluctuations in such symptoms as anorexia and asthenia. Cat BR was such an animal. He was frequently on the border line. Some extracts caused

improvement while others allowed him to drop back. Apparently the extracts varied in potency.

If the animal reached the stage of prostration the injection of cortical extracts was useless in most instances (cat ET).

From protocol of cat BR. Weight 2.99 kgm. at first adrenal removal. Weight (twenty-four days later) 2.44 kgm. at second adrenal removal.

*1 day* after removal of second adrenal, blood urea, 81.2 mgm. per 100 cc.

First eight days ate 25 to 65 grams of meat daily.

Blood urea between 86 and 128 mgm. throughout the experiment.

*27 days.* Weight 2.48 kgm. Excellent condition.

*28 days.* Feces slightly bloody. Vomited.

*33 days.* Slightly weaker. Blood sugar 74.6 mgm. per 100 cc.

*42 days.* Weight 2.41 kgm. Not so active as usual but ate well.

*45 days.* Alert. Blood sugar 90 mgm.

*54 days.* Bloody feces. Ate 50 grams of beef then vomited.

*58 days.* Condition excellent, unusually active.

*59 days.* Had vomited food which consisted of beef, egg yolk, butter and orange juice. Gradually growing weaker. Convulsions occurred at intervals for hours. Died the next day.

*60 days.* Weight 2.2 kgm.

*Autopsy.* Gingivitis, teeth practically all loose. Formerly pink skin dirty grey. Hair can be pulled out in handfuls anywhere over body. Careful exploration failed to disclose accessory adrenals. Gastric ulcer, 3 cm. in diameter.

*Metabolism.* In figure 1 are given the data of seven cats on which observations were made both before and after the second adrenalectomy, either in sufficient number or at wide enough intervals to believe that the normal and post-operative metabolism is described with some accuracy. Of the cats in this series U is the only one whose really *normal* metabolism is shown; in this cat, both adrenals were taken out so closely together that the metabolic level just preceding the second operation was not determined. In the remaining cats of this series sufficient time was allowed to elapse after the removal of the first adrenal for the animal to show a good recovery from the operation; it was then attempted to establish the level of the metabolic rate before removing the other gland. This metabolic rate will be referred to as *normal*.

From previous work of a similar nature (2) we should expect the metabolism to fall continuously in the majority of cases from the time of removal of the second adrenal. In our experience, however, this occurs in only one cat (ER) out of seven. In the other six, the metabolism remains within "normal" limits for periods ranging from 7 to 29 days. We interpret this to mean that the cortical extract which is injected after the removal of the second adrenal is sufficiently efficacious to postpone the onset of the decline in metabolism which must otherwise be expected.

*Loss of weight.* Treated adrenalectomized cats rarely gained weight, even those living for several weeks. They would lose and then maintain the weight at a lower level for some time (cats BR and ET).

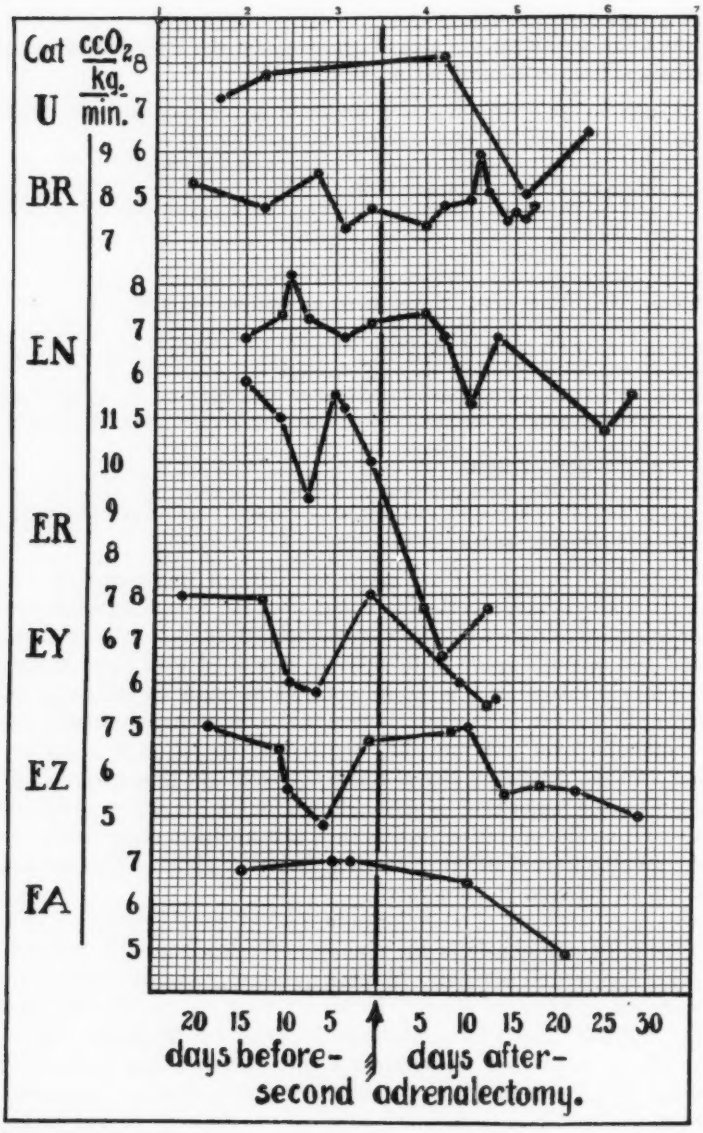
Cat
U
BR
EN
ER
EY
EZ
FA
cc O2 / kg. min.
20 15 10 5 5 10 15 20 25 30
days before-second
days after-adrenalectomy.

Fig. 1

From the protocol of cat ET. Weight at removal of second adrenal, 2.3 kgm. Injected daily with extract from about 10 grams of cortex in 5 to 10 cc. of fluid. Ate from 50 to 100 grams of meat daily.

| *Days after operation* | |
|---|---|
| 2 | Condition excellent. |
| 5 | Slight gingivitis. |
| 7 | Coughs and sneezes. Does not appear so alert. |
| 14 | More alert and active. Blood urea 126.4 mgm. per 100 cc. |
| 17 | Condition excellent. Blood urea 98 mgm. Weight 2.36 kgm. |
| 21 | Blood urea 78 mgm. |
| 31 | Blood urea 106 mgm. |
| 47 | Excellent condition, runs for food. Weight 2.03 kgm. Blood urea 76 mgm. Frequently killed and ate rats offered to it. |
| 69 | Extract discontinued. Weight 2.03 kgm. Blood urea 102 mgm. |
| 73 | Now eats only 40 to 60 grams daily where it ate 80 to 100 grams before. |
| 79 | Ate 60 grams. |
| 80 | Refused food, appeared ill. A few hours later animal lying on side breathing slowly. Injection of cortical extract without effect. Autopsy: Weight 1.88 kgm. Slight bronchial infection. Three gastric ulcers. No accessory cortical bodies. Epidermis of tongue, mouth and lips peels easily. Survived 80 days. |

*Resistance to infections.* Adrenalectomized animals which have their survival period prolonged by extracts often contract an infection in the late stage of their survival. This may be mild or it may be so severe as to cause the death of the animal. We have found the respiratory tract to be most often affected in this respect. It seems that these treated animals have a lower resistance than do normal animals although higher than untreated adrenalectomized animals.

*Resistance to operations.* Treated adrenalectomized animals although apparently in excellent condition do not seem to be good operative risks. A cat which appeared in excellent condition eleven days after complete adrenalectomy was carefully anesthetized with ether and the kidney exposed on one side extraperitoneally. It died as soon as the kidney was touched. This would be rare for a normal cat.

*Response to cold.* It is well known that in the late stages of acute adrenal insufficiency, the rectal temperature falls (Elliott). In the more chronic condition of our animals the rectal temperature may be fairly well maintained until a few hours or a day or two before death at ordinary room temperature. Again it may show fluctuations. Cat OY illustrates the latter.

*Rectal temperature cat OY*

| | DAYS AFTER COMPLETE ADRENALECTOMY | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 19 | 34 | 36* |
| Degrees Centigrade..... | 39.0 | 39.2 | 38.0 | 39.4 | 38.4 | 39.2 | 39.4 | 38.7 | 38.2 | 38.9 | 38.1 | 34.2 |

* Three hours before death.

The influence of the surrounding temperature upon the rectal temperature is shown by cat I.

*Cat I (survival 11 days)*

| | DAYS AFTER COMPLETE ADRENALECTOMY | |
|---|---|---|
| | 4 | 8 |
| Rectal temperature, °C | 39.8 | 37.9 |
| Room temperature, °C | 32.5 | 28.4 |

Not only did treated adrenalectomized cats seem to react poorly to cold but if exposed to a cooler room than that to which they were accustomed they readily developed "snuffles."

These observations led us to keep our animals in a room with a constant temperature and in order to favor animals recovering from an operation and those in the later stages of adrenal insufficiency a fairly high temperature was maintained, viz., 27 to 28°C.

*Recovery from exercise.* Daily injections of cortical extract not only prevent the onset of asthenia but raise the resistance to fatigue. Animals so treated stand the ordinary manipulation attendant upon the drawing of blood and injections. A certain amount of mild exercise as walking about the room likewise is tolerated. But vigorous exercise always tires the animal more than it would a normal animal. Sometimes such exercise leads to acute symptoms terminated in a few days by death. The reaction undoubtedly depends upon how near the animal is to the border line.

A live adult rat was placed in each of the cages of two treated adrenalectomized cats in good condition (25 and 29 days respectively after complete adrenalectomy). The first cat caught his rat with little difficulty. The rat happened to escape from the second cat. They both dashed about the cage before the rat was caught. Each cat became very tense and excited but the second unusually so. Moreover he went through much more vigorous exercise. The cats then ate the heads of the rats which entailed considerable labor. The first cat showed signs of weakness the next day and ate very little. On the second day he was still weaker; dying on the fourth day.

The second cat was very unsteady on his feet the day after exercise and died on the second day.

*Indications of a kidney-adrenal relationship.* The work of Marshall and Davis (3) on blood urea in acute adrenal insufficiency indicated that the cortical hormone was necessary for normal kidney function. Work which we have already published (4) confirms this interpretation. We are now reporting further observations which support this idea.

*Blood urea and diet.* Early in our experiments we noticed that if a cat was allowed to eat all he wanted on the day after the removal of the second adrenal he sometimes died within a few hours or he might eat very little on succeeding days dying within a few days. We believed that overeating hastened the onset of serious symptoms and death. This belief was confirmed by numerous observations on cats that had survived complete adrenalectomy for several days. They were in excellent condition and were allowed to eat all that they wanted of some kind of food which they especially liked but had not tasted for days. Thus cats living mostly on canned salmon and milk would overeat when offered fresh beef.

The blood urea in these animals would become very high and the symptoms of adrenal insufficiency exacerbated. Some did not recover but died in a few days. Others might recover, the blood urea dropping and the appetite returning. Liver especially produced this reaction. Cat EN shows the effect of feeding beef. These animals were all being injected with cortical extracts.

From the protocol of cat EN. Before the removal of the second adrenal, blood urea ranged from 43 to 80 mgm. per 100 cc.

5 days after complete adrenalectomy, blood urea 81 mgm., 37 grams of salmon eaten.

6 days after, 100 grams of fresh lean beef eaten.

7 days after, food refused. Blood urea 206 mgm.

8 days after, 5 grams of salmon eaten. Blood urea 217 mgm.

9 days after, 30 grams of salmon eaten.

10 days after, 68 grams of salmon eaten. Blood urea 122 mgm.

11 days after, 130 grams of salmon eaten. Blood urea 78 mgm.

Blood urea thereafter fluctuated between 90 mgm. and 153 mgm. Died at 31 days from occlusion of bronchioles with pus. No accessory adrenals found.

*Kidney damage.* If the kidneys are functioning at a lower level due to adrenal insufficiency, removal of kidney tissue might be expected to hasten the onset of symptoms just insofar as the kidney contributes to the condition.

In cat BM a wedge-shaped piece of the right kidney was removed at the time of the first adrenal operation. Seven days later the left adrenal was removed. This cat had normally shown a high blood urea (85 to 102 mgm.) at times before any operation had been performed. It was not surprising therefore to find it consistently high after complete adrenalectomy. It was receiving injections of the early NaCl precipitate preparation twice daily. On the fifth day it was eating well (150 grams of salmon), although the urea was 121.5 mgm. But on the seventh day it refused food and took nothing except milk until death. On the tenth day the blood urea jumped to 196 mgm. On the day of death (twelfth day) the urea was 248 mgm.

The urinary bladder was filled with milk-white urine due to the large amount of lipoid present. This is the only cat in which such a quantity of lipoid was present in the urine. As already pointed out (4, 5) a considerable increase in lipoid was commonly found in the kidneys of adrenalectomized cats.

*Skin alterations.* Many unsuccessful attempts have been made to find an increase in pigmentation in experimental adrenal insufficiency. Nor have we succeeded in finding a definite change of this sort. However, we have noted in a few instances where the hair was white or light colored in an area frequently shaved for injection that after several days this skin became dirty grey (cat BR). This was after fresh shaving. The area was formerly pink when freshly shaven. No pigment could be detected.

A marked change did occur in some animals. The hair began to loosen more and more until it finally was pulled out easily in handfuls. This was not entirely accounted for by the warm room because many animals both normal and adrenalectomized did not show it at all or only to a very slight extent.

On the other hand growth was occurring because new hair was replacing the old.

In connection with the search for pigment we must mention our findings in cat BQ (q.v.) which lived for 300 days, after the removal of both adrenals.

The most striking post-mortem finding was the dark chocolate color of the fat in the mesentery, omentum and subcutaneous tissue. The subcutaneous fat was darkest in the lumbar region and least in the cervical region. It was uniformly dark in the omentum and mesentery. The retroperitoneal and orbital fat were uncolored. The coloring matter of this fat was soluble in 5 per cent ammonium hydroxide or acetone. It was decolorized by standing a few hours in distilled water. It was insoluble in ethyl alcohol, methyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, carbon bisulphide and xylol.

We have noticed only one other cat showing brown fat. This cat survived 41.5 days after the removal of the second adrenal. The fat, however, was a light brown instead of dark chocolate brown. It's distribution was about the same as in the preceding animal.

*Thyroidectomized and gonadectomized animals.* Zwemer (6) found that thyroidectomy prolonged the life of adrenalectomized cats. We have tried the experiment of removing the thyroids, gonads and adrenals to see whether such animals when treated with cortical extract would respond differently than animals only adrenalectomized.

Five cats completely thyroidectomized for a month or more were adrenalectomized in the usual way. Two females and two males were gonadectomized at the first adrenal operation. The gonads were left intact in the

fifth, a male. These cats were injected daily with 3 or 4 cc. per kilo of an extract of the NaCl precipitate prepared according to the earlier method.

The cat with only thyroids and adrenals removed survived 17 days. Three of the others survived 10.5, 14.5 and 18 days respectively. These results indicate that thyroidectomy and gonadectomy do not affect the survival period.

The fifth cat deserves special consideration (see protocol BQ). He was injected as were the others for 67 days following complete adrenalectomy. During the first seventeen days the blood urea rose from 50 mgm. to 100 mgm. per 100 cc. At this stage he suffered from gingivitis and appeared somewhat lackadaisical. Then he improved so that he finally (67 days) appeared normal. The blood urea was then around 50 mgm. The extract was discontinued. He gained in weight until 4.2 kgm. was reached on the 106th day. He then gradually lost but there was no other apparent change until a few minutes before death when he was seized with convulsions. He then weighed 3.46 kgm. and had survived 300 days.

*From the protocol of BQ.* Thyroids removed one year before first adrenal operation. Testes and right adrenal removed. Weight 3.55 kgm. Left adrenal removed 17 days later. Weight 3.15 kgm. Extract of NaCl precipitate injected daily for 67 days.

*Two days* after last operation blood urea 49.2 mgm. per 100 cc. Beginning on the third day 75 grams of meat eaten daily. This amount increased to 120 grams daily from the eighth day and continued for weeks:

*17 days.* Blood urea 102.0. Weight 3.6 kgm. Appears somewhat lackadaisical. Gingivitis present.

*23 days.* Blood urea 67.2. Blood sugar 83.3 mgm. per 100 cc. Excellent condition. Appears almost normal.

*52 days.* Blood urea 41.4. Blood sugar 101.4. Strong, alert and pugnacious. Weight 3.95 kgm.

*67 days.* Appears to be normal. Weight 4.1 kgm. Extract discontinued.

*210 days.* Weight 4.0 kgm. Appears normal.

*224 days.* Weight 3.8 kgm.

*255 days.* Weight 3.6 kgm.

*300 days.* After having been allowed to run at large in the laboratory for a few hours (a customary practice) died in convulsions. Weight 3.46 kgm.

*Autopsy.* Heart dilated; old endocarditis. Chronic passive congestion of liver, spleen and kidney. Lymph nodes in thorax and abdomen very large and dark brown. Fat dark chocolate brown (see "skin alterations" for details). One parathyroid but no thyroid tissue found. Stomach and intestine with no congestion or ulcers. Two accessory cortical bodies one weighing 22 mgm. and the other slightly less.

DISCUSSION. The injection of cortical extracts not only much prolongs the survival period of adrenalectomized cats but postpones for a considerable time the development of symptoms.

Of preceding work on the metabolism of adrenalectomized animals that of Scott (2) is, perhaps, most closely related to our own. He also worked

with cats and produced varying degrees of adrenal insufficiency by ligation or freezing; which, assuming our extract to be potent, is probably comparable to total removal followed by extract injections. If the adrenal injury was sufficient to cause any effect at all, Scott was able to distinguish two types of results: 1, severe, but not fatal injury caused an increase in metabolism above pre-operative levels, followed by a return to normal; 2, lethal injury: the metabolism might remain equal to, or slightly exceed the pre-operative level for 4 to 5 days but then, and in most of the cases, progressively from the time of injury, fell to subnormal values until death.

Our own animals are similar to Scott's second group as to condition and, on the whole, confirm his observations as to results. The chief difference between his work and ours seems to concern, mainly, the proportions in which the possible effects occur. In six out of eight cases that terminated fatally, Scott observed a continuous decrease in metabolism after the adrenal injury; in only 2 cases was this decline postponed for a few days during which the metabolism remained within or slightly exceeded normal limits. Perhaps one of the strongest bits of evidence for the efficacy of our extract is the fact that in our animals this proportion is reversed. Only one out of seven of our cases shows a progressive decline in metabolism following removal of the second adrenal. In the other six cases the post-operative metabolism remains within the actual normal limits or varies less than 10 per cent therefrom from periods ranging from 7 to 29 days. In other words, although the extract is not capable of completely replacing the removed glands it does have a very measurable effect in postponing the fall in metabolism that could be expected without its use.

Loss of weight is perhaps to be expected. Often there is a considerable weight loss following the first adrenal operation. Of the whole series only one animal gained in weight after the second adrenal operation. This animal (BQ) possessed two accessory cortical bodies. Perhaps it would be necessary to have the cortical hormone present throughout the life of the animal if he were to gain in weight. Injected extracts may furnish the hormone for only a few hours at a time. The loss in weight in some of our animals may be a direct effect of the extract itself due to the presence of foreign protein or other deleterious substances. We have found that such injections daily into normal white rats caused a failure to gain as compared with uninjected controls for about 20 days (unpublished results).

The failure of animals, suddenly deprived of their last adrenal, to resist cold, infections and fatigue although injected twice daily with cortical extract is not surprising. However, these animals fare much better than similar ones untreated. Again the condition might be explained by the absence of a hormone supply for several hours out of the twenty-four. This might have a cumulative effect.

If the histological changes of the adrenal cortex which are found accom-

panying infections (7) and fatigue (8) indicate overactivity of the cortex there is need of an increased amount of hormone under these conditions. Such a need could be met by the intact adrenal but hardly by injection. That is, the intact adrenal could render a quick response more in proportion and delivered as needed while injection is largely guesswork.

The function of the cortical hormone in relation to the kidney is an interesting question.

Much evidence has accumulated which points to a direct relationship between the kidney and the adrenal (see references in (4). Gunn (5) working with our material has shown that lipoid nephrosis is typical of adrenal insufficiency in the cat.

According to E. M. and L. L. MacKay (9) the adrenals of patients who die in the uremia of hemorrhagic Bright's disease are usually larger than those from patients dying from other causes. They found that the adrenals of rats, rendered uremic by double nephrectomy, became heavier by 46 to 65 per cent as compared with controls. The increased weight seemed to be due in part to lipoid substances. Histological examination indicated that the increase was in the cortex.

Our experiments show that control of the diet is extremely important in adrenal insufficiency. This may be accounted for in part by lowered renal function. It seems therefore that minimal quantities of food and choice of those which make the least demand upon the kidneys, is desirable.

Indirectly this failure of the kidney will react on other tissues of the body. The water balance and the proportion of excretory as well as useful substances may be upset. This in itself might lead to grave consequences.

However, the cortical hormone may have a direct effect on other tissues. Whatever the effect, the changes produced are not prompt like insulin or parathyrin.

## SUMMARY

A condition simulating chronic adrenal insufficiency has been produced by the daily injection of extracts of the adrenal cortex into completely adrenalectomized cats.

In general, the symptoms are those of acute insufficiency except that they tend to develop more gradually. In a majority of the animals treated with extract, the metabolism remained within normal limits for periods ranging from 7 to 29 days after removal of the second adrenal. The animal never gained weight and usually lost long before the onset of terminal symptoms.

These animals were less resistant to cold and infections. They fatigued more readily than did normals.

Overeating caused the blood urea to rise, and brought on symptoms of adrenal insufficiency.

In a few instances the skin changed from pink to dirty grey in color. A chocolate-brown subcutaneous fat was found in a cat which survived 300 days.

Thyroidectomy and gonadectomy apparently did not modify the survival period of treated adrenalectomized cats.

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# THE ENERGY CHANGES OF SKELETAL MUSCLE ACCOMPANYING CONTRACTION UNDER HIGH PRESSURE[1]

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Received for publication June 27, 1928

Recent observations (1928) have shown that cardiac tissue exhibits a striking augmentation in activity during compression through a fluid medium. Evidences of stimulation are apparent in the increased rate, shorter conduction interval, and in the marked increase in contractile force exerted by the heart. It is the purpose of the present communications to report the results of a further study designed to throw light on the mechanism by which these changes are produced.

METHOD. In order to simplify the conditions and make more accurately controlled observations possible we have substituted skeletal muscle and the isometric lever for the cold-blooded heart and membrane manometer used in our earlier experiments. The same general technic for applying pressure and recording muscular activity has been continued. A sensitive torsion wire isometric lever of high frequency, which is an adaptation of the type described by Fulton (1925) was mounted within the pressure chamber before a glass window. A small mirror placed at the axis of rotation of the wire permitted the reflection of a beam of light from an external source through the window to a camera. The muscle—gastrocnemius from the frog—was mounted by tying the tendonous end to the arm of the lever, while the knee joint, to which the other end of the muscle remained attached, was fixed to a special clamp. This clamp was part of a rod which was threaded to fit a large nut resting on a fixed support. By turning this nut the distance of the muscle clamp from the lever arm could be varied thus bringing to bear upon the muscle any desired initial tension. For the purpose of accurately controlling the initial tension while the muscle was sealed within the pressure chamber an arrangement of gears was constructed by means of which the adjusting nut could be controlled by a thumb screw outside the chamber. Details of the recording and regulating mechanism are illustrated in figure 1.

Measurements of temperature occurring as the result of functional activity of the muscle were made through a small thermopile of the type

[1] A preliminary summary of this work appeared in the proceedings of the American Physiological Society, THIS JOURNAL, 1928, lxxxv, 358.

employed by Hartree and Hill (1920) and by Fenn (1923), mounted in the chamber in close relation to the position occupied by the muscle. In constructing the thermopile, 50 turns of constantin wire were wound on a flat ivory disc, which was later partially silver plated so as to give 50 "hot junctions" on each side of the thermopile and a corresponding number of "cold junctions" at the edges. When completed the thermopile had a resistance of 36 ohms. The galvanometer employed was of the d'Arsenval type, manufactured by Leeds and Northrup, with a critical damping resistance of 40 ohms, and a sensitivity of about 10 millimeters per micro-

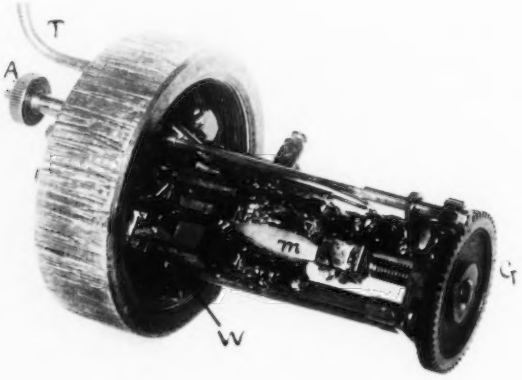


Fig. 1. Arrangement of the apparatus attached to the head of the pressure chamber. The muscle, *M*, is connected to the torsion wire lever system which has a mirror affixed to it before the glass window, partially shown at *W*. The initial tension is regulated by the adjusting screw, *A*, which acts through the gears shown at *G*. The pressure is conducted to the chamber by the brass tubing, *T*. Two sets of insulated wires are passed through the cylinder head, one pair serving to conduct current to the stimulating electrodes and the other pair leading from the thermopile.

volt on a scale placed two meters from the mirror. This combination was of such sensitivity that the single twitch of one muscle resulted in a deflection on the scale of about 20 millimeters. The experimental conditions under which we found it desirable to work, i.e., with the gastrocnemius muscle immersed in fluid under high pressure and stimulated through its nerve, made it impracticable to calibrate the galvanometer excursions in terms of absolute units of heat, and, therefore, the heat production has been recorded only in terms of the galvanometer deflections. This is entirely satisfactory for the present investigation since we have been concerned only with the relative effects of pressure on the initial heat production and in every

preparation observations were made at atmospheric pressure as well as at the higher pressures.

In many experiments, in order to increase the galvanometer excursions, two muscles were connected with the lever system their positions being such that they rested against opposite sides of the flat thermopile.

Stimulation was applied through the sciatic nerve which was placed across a pair of electrodes within the chamber, and these in turn were connected by insulated wires to the secondary of an induction coil. In those experiments in which a tetanic stimulation was employed, a timing device was placed in the secondary circuit and the muscle was usually excited for 0.1 second.

TABLE 1

*Results of the application of pressure (882 lbs. per sq. in.) to skeletal muscle*

| EXPERIMENT | PRE-PRESSURE | | PRESSURE | | POST-PRESSURE | |
|---|---|---|---|---|---|---|
| | Heat | Tension | Heat | Tension | Heat | Tension |
| 7 | 2.4 | 60.5 | 3.2 | 86.0 | 1.6 | 61.5 |
| 8 | 2.9 | 60.5 | 3.2 | 71.0 | 2.8 | 56.0 |
| 9 | 3.2 | 48.5 | 4.2 | 56.0 | 3.0 | 45.5 |
| 10 | 2.1 | 48.0 | 3.0 | 59.5 | 2.2 | 45.0 |
| 11 | 2.6 | 36.0 | 3.1 | 52.0 | 2.5 | 36.0 |
| 13 | 2.3 | 41.0 | 3.6 | 67.0 | 1.8 | 48.0 |
| 14 | 2.2 | 40.0 | 2.6 | 58.0 | 1.8 | 42.0 |
| 16 | 3.1 | 75.0 | 3.8 | 88.0 | 2.8 | 72.0 |
| 17 | 2.3 | 56.0 | 3.3 | 73.0 | 2.1 | 60.0 |
| 18 | 1.7 | 41.5 | 2.0 | 49.5 | 1.7 | 39.5 |
| 19 | 2.0 | 37.0 | 3.1 | 58.0 | 1.7 | 38.0 |
| Average... | 2.43 | 49.45 | 3.19 | 65.27 | 2.18 | 49.40 |
| Percentage increase..... | | | 31.2 | 31.9 | | |

In our earlier experiments with cardiac tissue and with some of the present series, Ringer's solution was used to fill the chamber and thus act as the medium for the transmission of the pressure to the tissue. Great difficulty was experienced in keeping the salt solution from short circuiting the thermopile or connections under the comparatively high pressures employed, so that in later experiments where measurements of heat production were desired liquid petrolatum was substituted. Compressed nitrogen was used as the source of pressure, which was transmitted through an intermediary chamber to the fluid surrounding the muscle, details of which are given in our previous paper.

The usual procedure in carrying out an experiment was to mount the preparation, close the chamber tightly, make the necessary connections with the source of pressure, etc., and then to wait for about half an hour

for the temperature to become uniform. A constant initial tension, usually about 50 grams, was maintained throughout the course of the experiment. This necessitated an occasional adjustment to compensate for stretching occurring as a result of contraction. A series of control observations was always made before pressure was applied, i.e., the tension changes as the result of a stimulus were photographed and simultaneous observations made of the galvanometer deflection. Observations were repeated after the application of the desired pressure, the highest employed being 1000 pounds per square inch.

THE EFFECT OF PRESSURE ON THE HEAT-TENSION RATIO. In the first series of experiments the effects of sixty atmospheres pressure on the response of a pair of muscles to a single stimulus was studied. Simultaneous determinations of the heat production and tension were made, and these figures have been summarized in table 1. A series of control observations was made before the application of pressure and again after its release. The pressure was usually allowed to act for about half an hour during which time measurements were made at five-minute intervals. The figures in the table represent for each experiment the average of from three to ten observations. In the first eight experiments the muscles were immersed in Ringer's solution and in the remainder in liquid petrolatum.

The invariable result of the application of this degree of pressure is an immediate increase in both the tension and heat accompanying the single twitch. The individual experiments vary considerably in the amount of the stimulation. Difficulty was experienced in obtaining a perfectly steady galvanometer in those experiments carried out in the salt solution, due to the tendency at high pressures for defects in the insulation to appear, and for this reason the heat measurements were subject to some variation. However, taken in conjunction with later experiments in which oil was used, the average value of a 31.2 per cent increase in heat production is probably not far from the true figure.

In the same preparations the pressure resulted in a corresponding increase in tension developed, the average for eleven experiments being 31.9 per cent. In other words, it may be stated that the application of a pressure of sixty atmospheres to a muscle caused an increase of about one-third in the work done in consequence of a single maximum shock, without changing the efficiency of the muscle to an appreciable degree.

The onset of stimulation probably appears simultaneously with the application of pressure; certainly it is fully developed within a second after the pressure is increased. If pressure is applied while the muscle is undergoing rhythmic contractions in response to regularly repeated stimuli at a rate of 30 to 60 per minute, the next succeeding contraction always shows a fully developed increase in the tension record. Figure 2 shows a typical example of the influence of pressure on a single muscle which was being

stimulated 58 times per minute. The figures in table 1 were obtained as the result of stimuli applied some minutes after subjecting the muscle to pressure, but the degree of stimulation did not change appreciably during the pressure period. In one instance a pressure of sixty-five atmospheres was applied to a muscle, with a resultant increase in the tension developed of 48 per cent. Two hours and twenty minutes later, the pressure remaining constant during the interval, the single twitch still developed a tension 44 per cent higher than the pre-pressure value, and it at once fell to the initial level when the pressure was released. If the frequency of stimulation is sufficiently great or if the muscle is in a partially fatigued condition, as it was at the time figure 2 was recorded, there is some falling off in the

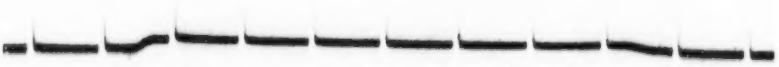

Fig. 2. Tension records of a muscle stimulated at the rate of 58 times per minute, showing the immediate change in response following the application and removal of a pressure of 1000 pounds per square inch. The small shift upwards in the position of the base line indicates the period during which the pressure was applied.[1]

[1] The shifting of the base line does not represent a change in the initial tension, but is due to action of the pressure on the walls of the chamber, which causes the position of the attached apparatus including the lever system to be slightly changed.

maximum tension of the twitch during the application of pressure. In two experiments, where such a result has been observed, the contractions during the post-pressure period were continued for a long time without exhibiting a further fall and even showing a temporary improvement. This result is of interest in that it indicates a more rapid exhaustion from activity under pressure, and it corresponds with our previously reported observations made on rhythmically beating cardiac muscle.

THE RELATION BETWEEN PRESSURE AND THE DEGREE OF STIMULATION. Further experiments have been carried out to show the relationship between the degree of pressure and the resulting stimulation. As in the previous experiments a pair of gastrocnemius muscles were mounted in the pressure

chamber which was filled with oil. Pressure was applied gradually, i.e., it was added in increments of 100 to 200 pounds per square inch up to 1000 pounds, and then reduced in corresponding stages to atmospheric pressure. At each level of pressure a single stimulus was applied through the sciatic

Fig. 3. Records of the tension developed by a muscle stimulated with a single shock at five minute intervals under pressures varying between one and sixty-five atmospheres. In order to make the degree of stimulation more apparent short horizontal lines have been drawn at the level representing the tension developed for the control stimulation at atmospheric pressure.

nerves and the resulting tension and heat production recorded. The pressure was permitted to act for five minutes before an observation was made in order to permit the equalization of small changes in temperature. Immediately after the observation the pressure was raised or lowered to the next level to be studied.

A tension record obtained in such an experiment is reproduced in figure 3 which shows photographs of the single twitch under pressure, increasing in steps of ten atmospheres up to sixty-five atmospheres and then back to atmospheric pressure. In this experiment the tension developed under the maximum pressure employed is 44 per cent greater than the control value. Data from another experiment, which show the influence of pressure on both tension and heat production, are given in the chart reproduced as figure 4. This experiment is typical of the six others carried out and indicates that the greater tensions developed under high pressure are

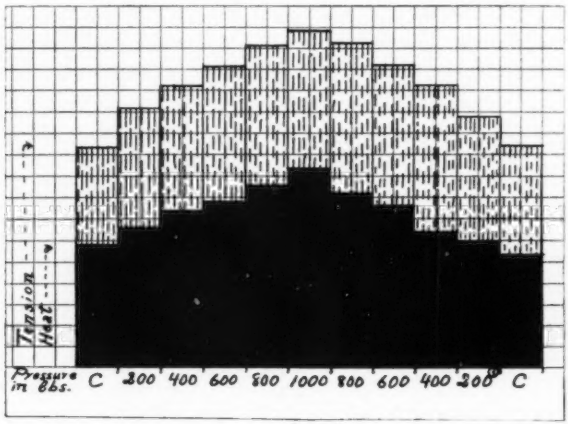


**Fig. 4. Chart showing a typical example of the relationship between the tension and heat production for a single twitch in a muscle exposed to pressures up to 1000 pounds per square inch. The observations were first made as the pressure was increased in stages, and were repeated as the pressure was released.**

[1] The value for the tension in the second observation at 200 pounds per square inch was lost. The level drawn in the chart represents the mean of the two adjacent values.

accompanied by corresponding changes in the initial energy set free. The ratio of heat to tension is constant; the extreme variations deviate not more than 10 per cent from the average and show no relationship to the pressure.

It is apparent from these experiments that the figures for the maximum tension developed by the muscle subjected to various pressures in response to a single stimulus all fall on an approximately straight line. That is, between atmospheric pressure and 1000 pounds per square inch, the magnitude of the stimulation is proportional to the pressure. We have not

studied the effects of pressures of more than 1000 pounds per square inch, and so can not say to what pressures this relationship holds. It must be limited, however, for Bridgeman (1925) has shown that egg white and ordinary muscle meat are coagulated by the action of pressures of the order of 75,000 pounds per square inch.

THE INFLUENCE OF PRESSURE ON THE SUSTAINED CONTRACTION. The last series of observations to be reported here is concerned with the influence of hydrostatic pressures on the energy changes in a sustained contraction. This has not been easy to demonstrate conclusively on account of the complicating factor of fatigue. This was particularly true in the experiments carried out with the muscle immersed in oil, when it worked under practically anaerobic conditions. In this environment the tension fell off after relatively few applications of the tetanic stimulus. A summary of the results

TABLE 2

*Figures give the percentage change in tension and heat for both single shock and tetanus induced by a pressure of sixty atmospheres*

| EXPERIMENT | TETANUS | | SINGLE SHOCK | |
|---|---|---|---|---|
| | Heat | Tension | Heat | Tension |
| 29 | +2.7 | −7.7 | | |
| 30 | −1.0 | +2.0 | +19 | +25 |
| 31 | −7.2 | 0 | +40 | +30 |
| 33 | −0.4 | | +52 | |
| 34 | −6.8 | −9.1 | +40 | +30 |
| 35 | +3.0 | +7.0 | +29 | +15 |
| 39 | −9.6 | −23.3 | +27 | +13 |
| 41 | +2.6 | +2.0 | +33 | +16 |
| Average | | | 34.3% | 21.5% |

of eight experiments is given in table 2. These data represent the changes in heat and tension resulting from the application of a tetanic stimulus lasting 0.1 second to a single nerve muscle preparation, before and during the application of a pressure of sixty atmospheres. It will be seen that the effect of the pressure on the tension and heat developed during tetanus is small and variable. In view of the evidence of fatigue present in all these preparations the results are not conclusive since this factor might mask a stimulation due to pressure should it exist. In carrying out these experiments several observations on tetanus were made at five minute intervals followed by a single stimulus before the application of pressure. The pressure of sixty atmospheres was then applied suddenly and after five minutes a single stimulus was again applied followed at intervals by a series of tetanic stimuli. The interposition of the single stimuli served as controls, and showed the usual stimulation under pressure: the heat

production being raised on the average 34.3 per cent, and the tension 21.5 per cent. Hill and his collaborators have observed that the heat-tension ratio is increased in fatigue, and this factor undoubtedly accounts for the relatively small increase of tension in this series of experiments. The muscle, immersed in oil and having been subjected to several short periods of tetanus before the effect of the single shocks was recorded, shows a loss in the power of developing tension.

In order to obtain further evidence relating to the influence of pressure on the tension of tetanus, additional measurements were carried out with the muscle immersed in oxygenated Ringer's solution, no attempt being made to measure the heat production. These muscles showed no deterioration during the period of the experiment, so that reliance may be placed on the results. In four preparations the application of sixty atmospheres pressure caused respectively a change of $-2.2$, 0, 0, and $+3.5$ per cent in the maximum tension. Evidently if pressure causes any increase in tension it is extremely slight as compared with the increased response observed with the single twitch.

Discussion. The action of pressure in increasing the heat liberated in response to a single stimulus, suggests a basis for the explanation of the increased mechanical work which the muscle performs under pressure. Our figures indicate that the muscle works at approximately the same efficiency under a pressure of 1000 pounds per square inch as it does at atmospheric pressure, and thus give no indication that the pressure stimulation is in any way related to changes favoring a shortening of the muscle or to an influence on the physical mechanism of contraction. We are therefore probably warranted in concluding that the stimulating action of pressure follows from an increased liberation of energy. In contrast to the observations on the single twitch it is an interesting fact that neither the total tension developed during a prolonged contraction (tetanus) nor the accompanying heat production are increased by pressure.

This difference in the action of pressure on the muscular response resulting from a single stimulus as compared with repeated stimuli indicates a fundamental difference in the conditions of energy conversion. That the two conditions involve differences in mechanism has been demonstrated by Hartree and Hill (1921) who find that the output of heat following a single stimulus or at the beginning of a series of stimuli is relatively enormous; the effects from succeeding elements of the stimulation decreasing until a steady state is reached. Moreover, these authors have shown that a rise of temperature decreases the heat produced as the result of a single shock, whereas it results in a marked increase in the steady flow of heat during tetanus, the temperature coefficient of the latter being 2.8. We thus have a reasonable basis for the explanation of this differential action, but it is

obvious that changes in pressure and temperature produce their effects through an influence on different parts of the system.

It is clear that physical limitations to the degree of shortening possible must be imposed by the structure of the muscle cell, and it seems likely that there is a corresponding limitation in the maximum force which a muscle can exert, beyond which further increases in the energy liberated would be entirely dissipated in the form of heat. That this may be an important factor in explaining the effects of temperature is suggested by the data given by Hartree and Hill (1921). They found for the single twitch that the ratio of heat to tension per unit length of muscle was constant at all temperatures, whereas temperature has a marked effect on this ratio in tetanus. Actual figures for the effect of a rise in temperature on the tension are not given, but the temperature coefficient for heat production was found to be 2.8 and it is stated that the efficiency of a muscle in maintaining a constant force was decreased on the average 2.3 times for a rise of ten degrees centigrade; there must, therefore, have been but a very small increase in the maximum tension developed as the result of a rather large increase in energy conversion. Taking into consideration only the data relating to the effect of pressure on the development of tension, it would be natural to assume that the absence of effect in our experiments in the case of tetanus is due to an already existing maximum contraction. That is, in a fresh muscle a tetanic stimulation might result in a tension approaching the maximum permitted by the contractile mechanism, so that any further energy conversion due to pressure would merely result in a lessened efficiency. In the single twitch, however, which only contracts to a fraction of that possible in summation, any increase in energy set free should be exhibited in an increase in the work performed. The above suggestion, however, is not adequate in that it does not take account of the evidence obtained from the measurement of heat during tetanus, which gives no indication of an increase under pressure.

Any explanation of the effects of pressure must be made in relation to the details of energy conversion and the mechanism of contraction, a subject on which we have very little exact knowledge. It is, however, interesting to relate them to the attractive hypothesis advanced by Hartree and Hill (1921) to explain muscular activity. Essentially this hypothesis assumes a reversible reaction between two substances, A (glycogen) and B (lactic acid), inside some narrow space whose boundaries are impermeable to B. Stimulation results in an increase in the permeability of these boundaries, allowing the active substance B to pass out to a region where it acts on the contractile mechanism. This upsets the equilibrium ($A \leftrightharpoons B$) and more A changes to B. It is the amount of B initially present which accounts for the sudden outburst of heat at the beginning of a series of stimuli, while the reaching of the steady state indicates an exhaustion of

the preformed active substance, the steady flow of heat now depending entirely on the conversion of more of this substance from A. On this basis Hartree and Hill ascribe the action of increasing temperatures in raising the level of heat production during a tetanus to an influence in speeding up the conversion of A to B, whereas in the case of the single twitch where energy liberation depends on the setting free of material already formed, a rise in temperature decreases the heat liberation due to the more transitory nature of the permeability change.

On the basis of the Hartree-Hill theory the stimulating action of hydrostatic pressures might be brought about either by an increase or prolongation of the permeability of certain membranes or by an influence in speeding up the conversion of glycogen into lactic acid. It is clear that the latter possibility is not the predominating one as the pressure influence is almost entirely on the initial phase or single twitch, little or no stimulation of tetanic activity having been demonstrated. On the other hand, if we assume that pressure has an influence on the initial phase, that is, in a prolongation of the interval of increased permeability following stimulation, our results accord well with the theory. The increased permeability would be followed, in the case of the single twitch, by a greater outpouring of energy, resulting in a greater heat production and a corresponding increase in the tension developed. Since, on this theory, the steady state level of energy liberation in prolonged activity depends on the speed of conversion of glycogen, the changed permeability should be without influence on this part of the process, which is what we observe in the absence of pressure stimulation in tetanus.

Apart from this or any other theory it appears that the stimulating action of pressure depends upon some influence on the liberation of the large amount of energy occurring in the initial contractions of prolonged activity and in the single twitch, and there is no evidence of an influence on the relatively small output of energy accompanying the steady state of a sustained contraction.

## SUMMARY

1. A method is described for studying the effects of hydrostatic pressure on the heat production and tension developed by an active muscle.

2. A pressure of sixty atmospheres results in an increase in the tension developed by a muscle in response to a single stimulus, averaging about 30 per cent. This augmentation develops immediately and persists as long as the muscle is compressed.

3. There is an increase in the initial heat production corresponding to the tension changes under pressure. There is thus no change in the efficiency of the muscle.

4. The degree of stimulation is proportional to the pressure in the ranges studied (from atmospheric pressure to 1000 lbs. per sq. in.).

5. Pressure results in but slight or no increase in the heat or tension developed as a result of tetanic stimulation.

6. The results are discussed in relation to the theories of the energy changes accompanying muscular contraction.

We desire to express here our thanks to Prof. Wallace Fenn to whom we are greatly indebted for placing at our disposal the facilities of his laboratory, and for giving to one of us instruction in the technic for myothermic measurements.

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# STUDIES ON AGLOMERULAR AND GLOMERULAR KIDNEYS

## II. Physiological

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Received for publication July 7, 1928

The present assigned rôle of the glomerulus in renal activity is, even with certain reservations, inadequately supported. Convincing proof of the extent of this inadequacy remains to be adduced. Experimentation that promised well at the outset has not yielded decisive results—especially that experimentation concerned primarily with glomerular versus tubular activity in the elimination of urine.

In an effort to discover more valid proof of the respective rôles performed by renal tubules and glomeruli, kidneys were sought in which there are no glomeruli as well as those in which there is considerable variation in glomerular development. Such kidneys are found in the highly evolved teleosts or bony fish. An account of these is given in the first or anatomical section (Edwards, 1928a).

Time was not available for physiological studies on all of the types of kidneys which were examined anatomically. The following anatomical types, however, were also studied with regard to the excretion of dye and the quantitative analysis of simultaneously taken blood and urine: *a*, kidneys in which there are no glomeruli at all; *b*, those in which there are very few glomeruli,—less than 0.1 per cent; *c*, those in which there are many glomeruli (60 to 70 per cent). A brief, preliminary account of the results of these studies has been published (Edwards, 1928b). Thus three genera of fish were studied in which the kidneys are entirely aglomerular, namely, Sygnathus, Siphonostoma and Hippocampus. These belong in the family Sygnathidae, sub-order, Lophobranchs. Of these three genera, Siphonostoma is the largest weighing 6 to 8 grams; Sygnathus, 4 to 6 and Hippocampus ("sea-horse") 2.5 grams. The average weight of the kidneys of the first two genera is 50 mgm. each and of the last, 25 mgm. In these three genera, regardless of species differences in other ways, the kidneys are entirely lacking in glomeruli and also in specific arterial vascularization.

Of the fish the kidneys of which contain very few glomeruli, only one genus and two species were studied, namely, Lophius piscatorius and

bodegassa. This genus belongs in the family Lophiidae, sub-order, Pediculati. The body weight of Lophius is 2 to 3 kgm. and its kidneys weigh 2 to 3 grams each. The kidneys are well vascularized arterially and venously. Despite the presence of arterial vascularization, only a very few glomeruli develop. Their relative functional importance must therefore be slight. The vast majority of tubules are tapering and closed at their apices, i.e., aglomerular.

Of the fish the kidneys of which contain approximately 60 per cent of glomerular-connected tubules, only one genus and species was studied physiologically, namely, Muraena helena, in the family Muraenidae, sub-order, Apodes. The body weight of Muraena is 2 to 3 kilos and its kidneys weigh 2 to 3 grams each.

The renal tubule of all these fish shows the equivalent cytological features found in the proximal and distal convolutions of the higher vertebrates (figs. 1 to 7) and also a short segment, the equivalent of a collecting duct. In the kidneys of these fish no equivalent has been found of the loop of Henle as it occurs in the mammalian kidney, although the glomerular-

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Free-hand drawings of typical tubules illustrative of those found in the kidney of certain bony fish, of the frog, turtle, fowl and mammal. The cytological features of the various segments of these tubules were drawn under the same magnification from preparations made by the senior author. The drawings of the tubules of turtle, fowl and mammal are slightly modified from similar ones made by Huber (1917).

Fig. 1A. Camera lucida drawing of a tubule of Sygnathus acus (× 160) and transections of the equivalent of the proximal and distal convolutions of the mammalian tubule. (× 650). This tubule is sufficiently typical also of the tubules studied in the kidney of other Lophobranchs (pipe fish) and in that of the Lophiidae (Lophius piscatorius or bodegassa).

Fig. 1B. Transections of the two major segments of the tubule of Lophius.

Fig. 2. Drawing of a diverticulated tubule of Hippocampus guttulatus (seahorse) showing transections cytologically typical of the two major segments. (× 650)

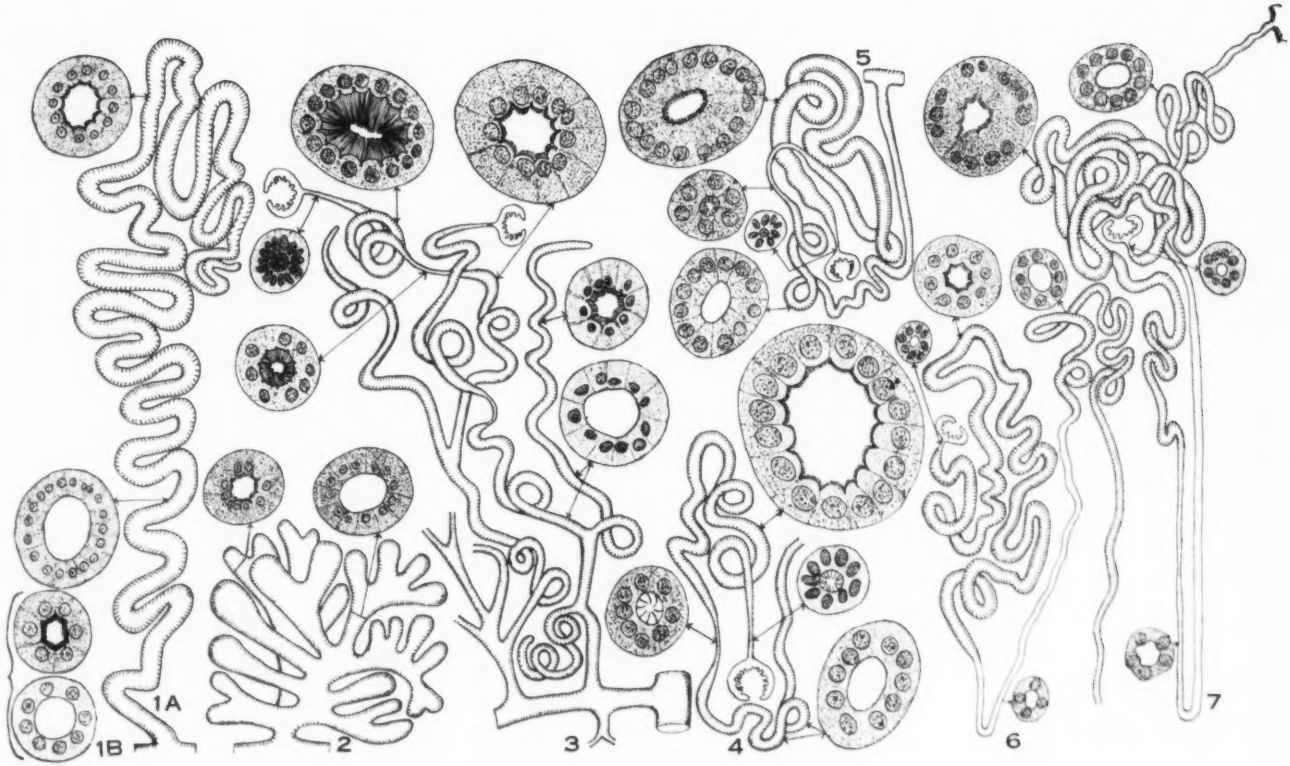
Fig. 3. Drawings of two types of tubules present in almost equal numbers in the kidney of Muraena helena. Note the greater morphological and cytological differentiation in the glomerular-connected tubule, here indicated as having a glomerular connection by the terminal expansion of the tubule and the indented inner layer of the glomerular capsule. This type of tubule is the morphological equivalent of the tubule of amphibia and mammalia, but cytologically the first convolution is unique. (× 650)

Fig. 4. Diagram of the tubula and camera lucida drawings of typical transections of the various segments as studied in the frog's kidney (Rana pipiens). (× 650)

Fig. 5. Similar representation of the turtle's kidney.

Fig. 6. Similar representation of the fowl's kidney. This type of tubule is depicted by Huber as being intermediate between that occurring in the turtle and mammal. According to Huber, at least 3 types of tubules occur in this kidney, i.e., reptilian, transitional and mammalian.

Fig. 7. Diagram of a mammalian tubule with drawings of transections of the human, renal tubule. (× 650)




Figs. 1–7

connected tubules of Muraena and of several other fish studied anatomically are comparable in external form to those of amphibia and reptilia.

Material: All of the fish used were obtained in excellent condition from the Gulf of Naples. They were brought when needed to the senior author's laboratory and placed in the aquarium with which each laboratory is supplied. Sea water was in constant circulation in this aquarium. All of the fish studied survive laboratory conditions well throughout the year, except Lophius. This fish does well in an aquarium during the winter and early spring. Later, it is either difficult to procure or dies within an hour or more after it is brought to the surface of the sea (or placed in an aquarium). Reference to this circumstance will be made again later. Muraena may bite the experimenter viciously unless proper care in manipulation is exercised.

I. THE ELIMINATION OF THE DYE, TETRACHLORPHENOLSULPHONEPHTHALEIN. *Method.* A. (For microscopic observations of the living kidney after intravascular and intraperitoneal injections. Experiments by the senior author alone.) *1.* Sygnathidae (Lophobranchs). A given fish was taken from the aquarium and placed in a 2 to 3 per cent solution of urethane. After anesthetization (15 to 30 minutes) it was placed on its back in a paraffined tray and pinned down. One straight pin was placed through the muscles of the body wall a short distance caudal to the anus and slightly eccentric to the midline of the long axis of the body. Another U-shaped one was placed cuff-like around the fish just posterior to the gills. A cavity was made in the paraffine so that the head and gills were inclined downwards. This permitted sea-water to bathe the gills and so keep the fish alive during the experiment while sea-water diluted one-half could be used more readily to cover the kidneys after dissection to expose them. This dissection was done under a binocular microscope with illumination provided for by an electric lamp and condensing lens. It consisted of a mid-ventral incision made near the anus and extending 4 to 5 cm. anteriorly. A narrow strip of body-wall was cut away longitudinally to the right and left of the first incision. This served to expose desired parts of the viscera.

Since no hypodermic needle was found small enough to be practicable for injecting the dye, a glass capillary pipette was drawn out over a microburner and filled by means of another capillary pipette which could be inserted into the first one. The pancreatic or hepatic vein was used through which to inject the dye.

Approximately 0.1 cc. of a 0.3 per cent solution of tetrachlorphenolsulphonephthalein was injected. This solution was prepared by adding the requisite amount of sea-water diluted one-half with distilled water. The dye was forced from the capillary by pressure exerted through a small rubber tubing held between the teeth at one end and connected with the capillary pipette at the other.

Exposure of the kidney permitting reliable microscopic observation of the tubules while the fish is alive is impossible, owing to the presence of the highly vascular walls of the swim or air bladder and peritoneum. Removal of these is too technical to be successfully accomplished in the short time these fish survive operative procedure. Therefore the experiments permitting such observations as are recorded here consisted in making the shortest incision requisite to expose the pancreatic or hepatic vein, followed at once by injection of the dye and temporary closure of the opening made by the incision. After varying intervals the fish was sacrificed, bladder and kidneys exposed and the presence or absence of dye recorded.

*2.* Lophiidae (Lophius: Experiment performed without anesthesia). The fish was placed on its back and a mid-ventral incision was made through the skin extending longitudinally 2 to 3 cm. between the pectoral fins. This served to expose a sufficiently large superficial vein extending along the surface of the pectoral muscles. Injection of 6 to 10 mgm. of dye through this vein was made by using a small hypodermic needle. After 15 to 45 minutes the abdominal cavity was opened and bladder and kidneys observed. The thick fibrous capsule surrounding the kidneys of Lophius made successful microscopic observations very difficult. Attempts at decapsulation were only partially successful. Readings of the amount of dye excreted in a given unit of time are approximate since a colorimeter was not available.

*3.* Muraena. No microscopic observations of the living kidney were made.

*Method.* B. (For experiments involving intraperitoneal injections only and without microscopic observations of the living kidney. Experiments performed in collaboration).

*1.* Sygnathidae. No anesthetic was used. The live fish was taken from the aquarium and held in the hands of an assistant. A ligature was placed very precisely around the fish so that it passed over and bound only the lower caudal half of the rim of the anus. This serves to close the urinogenital aperture without occluding the ureters. The latter is very easy of occurrence since the internal arrangement is such that a ligature occluding the anal aperture would also prevent the passage of urine through the ureters to the bladder. Successful cannulation of the bladder is not possible. Next, an intraperitoneal injection of 0.4 cc. of a 0.3 per cent solution of tetrachlorphenolsulphonephthalein was made and the fish replaced in the aquarium for 15 to 18 hours. Four or more fish were always used for a given experiment. Otherwise not enough urine could be obtained for accurate readings. At the end of this period, the fish were again taken from the aquarium, decapitated and urine taken from the bladder as follows: an incision was made near the anus and the bladder exposed. A test-tube of 5 cc. capacity was placed conveniently near on the operating table.

The fish was held close enough to the tube so that the tip of the bladder could be held over or inserted into the tube. Finely pointed scissors were used to cut the bladder at its tip, permitting the outflow of urine into the test tube.

Readings were also made of the amount of dye in the gall bladder. In order to obtain the bile for this purpose, sharp-pointed forceps were placed around the neck of the gall bladder, the neck cut and the bladder removed. It was held over a small test tube and contents collected.

*2.* Lophiidae (Lophius). Since at the time of these experiments (late spring) this fish could not be kept alive sufficiently long in the laboratory aquarium for successful results of intraperitoneal injections, a method was devised for performing these experiments at sea immediately after the fish were caught. Unfortunately, on the several occasions when this was attempted, no Lophii were available. This, however, is of no great consequence since the results of intravascular injections show clearly the ease with which this dye is excreted and apparently solely by the kidney.

*3.* Muraenidae (Muraena). Difficulties in handling this long, sleek, eel-like and highly muscular fish made necessary a brief period of light anesthesia. This was accomplished by placing it for 5 minutes or more in a bucket containing chloretone in sea-water. As soon as adequately anesthetized, it was laid in a tong-like device which served to keep the ventral surface uppermost. The invaginated wall of the urinogenital aperture was ligatured and a narrow slit made in the thick skin. A fine hypodermic needle could then be used to pierce through the muscles of the abdominal wall permitting intraperitoneal injection of the dye without subsequent leakage through the place of injection. The fish was replaced in the aquarium (where it recovered within 15 minutes from the effect of the chloretone) for 15 to 18 hours. It was then taken again from the aquarium, lightly reanesthetized, an incision made in the abdominal wall and the urine and bile taken from the urinary and gall bladder by means of a hypodermic needle and syringe.

The determination of the amount of dye present in the urine and bile was made by the comparative method of Michaelis after the addition to the standard and the solution to be tested of equal amounts of a 1 per cent solution of $Na_2CO_3$.

*Results.* 1. Intravascular or intraperitoneal injections,—entirely aglomerular kidney. When observed under the microscopic within 30 minutes after an intravascular injection of the dye the entire tubule is colored a light purple (the major part of the tubule is the equivalent cytologically of the proximal convolution of higher vertebrates) and a slight trace is seen in the urine. The appearance of the dye in the tubule may be appreciably longer following an intraperitoneal injection. It was planned originally to obtain comparative data concerning the rate of elimination of

dye following intravascular injections. This was not possible owing to very limited time, the large amount of preliminary anatomical study and the numerous technical difficulties inhering of necessity in this type of experimentation. However, certain broad features have been realized as will appear later.

*2.* Intravascular injections only,—kidney with very few glomeruli. Three experiments were performed. Approximately 15 to 20 per cent of the amount of dye injected was excreted through the kidney in 30 minutes. The tubules were colored a uniform purple when observed within an hour after injection of the dye.

TABLE 1

*The excretion of tetrachlorphenolsulphonephthalein following intraperitoneal injections*

The reading given below of the amount of dye excreted in the urine of Lophius piscatorius was made 30 minutes after an intravenous injection.

| GENUS OF FISH | KIND OF KIDNEY | URINE PER DAY | NUMBER INJECTED FOR UNIT SAMPLE | AMOUNT INJECTED | AMOUNT EXCRETED | | PER CENT EXCRETION | |
|---|---|---|---|---|---|---|---|---|
| | | | | | In urine | In bile | Kidney | Liver |
| | | *cc.* | | *mgm.* | *mgm.* | *mgm.* | | |
| Sygnathus..... | Aglomerular | 0.40 | 4 | 1.2 | 0.36 | 0.80 | 30.0 | 67.0 |
| | | | 6 | 1.2 | 0.22 | 0.96 | 18.0 | 80.0 |
| Hippocampus.... | Aglomerular | 0.2-0.3 | 8 | 0.6 | 0.18 | 0.40 | 30.0 | 68.0 |
| | | | 4 | 0.6 | 0.16 | 0.42 | 27.0 | 70.0 |
| Lophius..... | Practically aglomerular | 40–50 | 1 | 10.0 | 2.00 | 0.00 | 20.0 | 0.0 |
| Muraena..... | 50 to 60 per cent glomerular | 11–15 | 1 | 6.0 | 4.20 | 1.60 | 70.0 | 30.0 |
| | | | | 4.5 | 3.25 | 1.21 | 72.0 | 27.0 |
| | | | | 4.5 | 3.33 | 1.13 | 74.0 | 25.0 |

*3.* Predominantly glomerular kidney,—intraperitoneal injections without microscopic observation of the living kidney. The results of such injections are best shown in the accompanying table (table 1).

A point of interest between the percentage elimination of dye by the kidney and liver of three of the genera of fish studied (Lophius is not considered in this connection since successful intraperitoneal injections of dye were not made) is seen in the apparent reversal in the relative amounts of dye excreted by the kidney of Sygnathus and Hippocampus as compared with that of Muraena. Although the small fish (Sygnathus and Hippocampus) excrete a maximum of 30 per cent of the total amount injected, if the amount of dye per cubic centimeter of urine excreted is compared,

it is seen that the small fish excrete 3 times as much per unit volume as the large fish, Muraena. This statement refers to the total amount of urine eliminated per day. This difference seems clearly to be dependent upon the activity of the kidney in connection with its venous blood supply. A comparison of Hippocampus with Sygnathus (two genera of the same family) reveals a conspicuously higher output of urine and dye by Hippocampus when the difference in body or kidney weight between the two is taken into account. Hippocampus weighs 2 to 3 grams and Sygnathus 6 to 8. The average weight of the two kidneys is 50 and 80 mgm. respectively. The chief or sole difference in blood supply to the kidneys of the two fish is in the presence of an afferent caudal vein to the thick, caudal part of the kidneys of Hippocampus.

Furthermore, this idea is supported by a comparison of the output of urine of Lophius and Muraena. These two fish have at comparable stages of development the same body and kidney weight. The renal arterial blood supply is also comparable. In Muraena, however, about 60 per cent of the tubules are typically connected with glomeruli and are therefore considerably more differentiated than the aglomerular tubules of Lophius. Yet, the excretion of urine per day by Lophius is more than twice as much as by Muraena. The kidneys of Muraena, it would appear, have very definitely an anatomical advantage over those of Lophius for the excretion of urine. But neither the quantity nor constituency of the urine of Muraena seems to indicate this. There are to the kidney of Lophius 4 relatively large afferent veins whereas the afferent venous supply of the kidney of Muraena is chiefly that of the small intercostals. The further possible significance of the venous circulation of kidneys in general will be mentioned later.

II. QUANTITATIVE ANALYSIS OF BLOOD AND URINE TAKEN SIMULTANEOUSLY FROM FISH WITH AGLOMERULAR AND GLOMERULAR KIDNEYS. *Methods.* 1. Fifteen hours or more before collecting the urine and blood, the urino-genital aperture of each fish used was closed by a ligature. This ligature was made either entirely around the body at a very precisely determined place (Sygnathus and Hippocampus) or by one made subcutaneously around the inner wall of the aperture (Lophius and Muraena). At the end of 15 to 18 hours the fish was removed from the aquarium in which it had been replaced after making the ligature and subjected to the following procedure: For the small fish, Sygnathus and Hippocampus, the head and gills (the latter after cutting away the gill flaps) were washed in distilled water and the head cut off a short distance cephalad to the heart. Blood sufficient for one or more analyses was caught in a small test tube and defibrinated. For the analyses of the blood and urine of Sygnathus, 8 to 10 fish were used at one time in order to obtain enough

of each for the analysis of one or more constituents; for Hippocampus,—50 to 100, depending on their size and other factors.

Of the larger fish, Lophius and Muraena, a given specimen provided enough blood and urine for analysis without recourse to mixing samples as required above. Blood from Lophius and Muraena was taken as follows: The heart and truncus arteriosus were exposed, the latter ligated and cut, the cut end held over a test tube and truncus slit caudal to the ligature, the fish being held meanwhile tail up. Immediately after taking the blood, the urinary bladder was exposed and urine taken by using a hypodermic needle and syringe or (for the small fish) by inserting the tip of the bladder into a small test tube and slitting it with sharp-pointed scissors.

2. The analyses were made by the following methods: a. Non-protein nitrogen,—micromethod of Bang. b. Urea,—method of Condorelli (1922). c. Amino acid nitrogen,—Van Slyke. d. Creatine, creatinine and ammonia,—micromethod described by Pincussen (1923). e. Sodium and potassium,—micromethod of Kramer and Tisdall modified by Condorelli (1927). f. Calcium and magnesium,—micromethod of Condorelli (1925). g. Chloride,—micromethod of Bang modified by Condorelli (1926). h. Phosphate,—micromethod of Bell and Doisy.

*Results.* The following table (table 2) contains the analyses of blood and urine of four genera of fish represented in 3 distinct and widely differing families. Data, similar as far as possible, have been compiled for representative genera in the higher vertebrate phyla.

Analysis of table 2. 1. Constituents of the urine of the various animals grouped as lower or higher with respect to their concentration in the blood.

| | CONSTITUENT | URINE |
|---|---|---|
| Sygnathus | Na, Cl, K, P | Lower |
| | Ca, Mg | Higher |
| Hippocampus | Urea | Lower |
| | Cl | Higher |
| Lophius | Urea, Na, Cl, K | Lower |
| | $NH_4$, creatinine, Ca, Mg | Higher |
| Muraena | Na, K, P | Lower |
| | $NH_4$, creatine, creatinine, Cl, Ca, Mg | Higher |
| Frog | Uric acid | Lower |
| | Urea, $NH_4$ | Higher |
| Fowl | Na, Cl | Lower |
| | Uric acid, creatine, creatinine, P | Higher |
| Man | All (except sodium) | Higher |

TABLE 2

*A comparison of the results of quantitative determinations of urinary constituents in the blood (defibrinated unless otherwise noted) and urine of four genera of teleostean fish*

The first reading of each constituent as given in their respective columns is the most accurate and comparable. Where the determination of a blood constituent is not followed by that of the urinary taken at the same time from the same fish, the space is filled with dashes. Averages are intentionally not given. The determinations of frog urinary constituents are those of Vander Heyde (1921) and Toda and Taguchi (1913); of reptile,—Lewis (1918); of the fowl,—Mayrs (1924), Bock (1917) for amino acid N, of human,—Folin (1905) and Hawk (1926). An asterisk over a certain constituent indicates that whole blood was used.

| CONSTITUENT | AGLOMERULAR KIDNEY SYGNATHUS | | AGLOMERULAR KIDNEY HIPPOCAMPUS | | SLIGHTLY GLOMERULAR KIDNEY LOPHIUS | | PREDOMINANTLY GLOMERULAR KIDNEY, MURAENA | | AMPHIBIA SUMMER AND WINTER FROG, RANA (2 SP.) | | REPTILIA 2 TURTLES A AND B* CHELONE MIDAS | | AVES FOWL† GALLUS DOM. | | MAMMALIA MAN HOMO SAPIENS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Blood | Urine | Blood | Urine | Blood | Urine | Blood | Urine | Plasma | Urine | Plasma | Urine | Plasma | Urine | Plasma | Urine |
| | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* |
| Non protein N | 95.00*<br>98.56*<br>61.04* | 130.20<br>224.00<br>—— | 291.25* | 291.20 | 51.00<br>66.50<br>57.80<br>52.30<br>51.00<br>48.00<br>45.00<br>——<br>109.00* | 168.00<br>217.00<br>98.00<br>197.33<br>——<br>——<br>——<br>207.00<br>—— | 108.30<br>128.94*<br>90.16*<br>—— | 102.00<br>——<br>——<br>74.00 | 17.30 | 20.60 | —— | 150.0 | —— | —— | 31.00 | 1,120.00 |
| Urea | 123.00 | —— | 264.50 | 246.00 | 56.21<br>100.00* | 35.33<br>—— | 89.40<br>——<br>50.63* | 89.00<br>53.00<br>—— | 35.00 | 38.70 | —— | 100.0 | —— | —— | 30.00 | 2,084.00 |
| Uric acid | —— | —— | —— | —— | —— | —— | —— | —— | 1.30 | 0.17 | —— | 63.0 | 6.0 | 197.0 | 2.00 | 25.87 |
| Ammonia | —— | —— | —— | —— | 6.80 | 44.69 | 7.11<br>5.60 | 28.14<br>—— | —— | 1.27 | —— | 26.5 | —— | —— | 1.00 | 59.50 |
| Creatine | —— | —— | —— | —— | 1.27 | —— | 2.03<br>1.08* | 16.80<br>11.40 | —— | —— | —— | 12.1 | 5.0 | 14.0 | 5.00 | —— |
| Creatinine | —— | —— | —— | —— | 1.49 | 42.59 | 1.01<br>1.20* | 30.02<br>23.38 | —— | —— | —— | 3.8 | 1.3 | 4.0 | 1.50 | 107.69 |
| Urea N | 56.73 | —— | 123.40 | 101.04 | 25.73<br>46.60 | 16.16<br>—— | 41.72<br>——<br>23.62 | 41.70<br>24.73<br>—— | 16.30 | 18.00 | —— | 46.7 | —— | —— | 13.00 | 972.00 |
| Uric acid N | —— | —— | —— | —— | —— | —— | —— | —— | 0.43 | 0.06 | —— | 21.0 | 2.0 | 65.7 | 0.70 | 9.09 |
| Ammonia N | —— | —— | —— | —— | 5.60 | 36.80 | 5.85<br>4.60 | 23.17<br>—— | —— | 1.05 | —— | 21.8 | —— | —— | 0.15 | 49.00 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Creatine N | —— | —— | —— | —— | 0.41 | —— | 0.66<br>0.35 | 5.43<br>3.68 | —— | —— | —— | 3.9 | 1.5 | 4.5 | 1.00 | —— |
| Creatinine N | —— | —— | —— | —— | 0.55 | 15.83 | 0.38<br>0.45 | 11.04<br>8.69 | —— | —— | —— | 1.4 | 0.5 | 1.5 | 0.60 | 40.56 |
| Amino acid N | —— | —— | —— | —— | —— | —— | 48.32 | 14.43 | —— | —— | —— | —— | 21.0 | —— | 7.00 | 11.00 |
| Undetermined N | ——<br>41.83 | ——<br>—— | 167.85 | 190.16 | ——<br>25.51<br>——<br>——<br>——<br>——<br>——<br>62.34 | ——<br>29.21<br>——<br>——<br>——<br>——<br>——<br>—— | 11.37<br>99.92<br>——<br>—— | 6.23<br>——<br>36.90 | 0.57 | 1.49 | —— | 55.2 | —— | —— | 9.25 | 38.35 |
| Sodium | 475.60<br>500.68<br>—— | 178.19<br>220.20<br>186.26 | 372.00 | 378.45 | 556.80<br>479.35<br>——<br>537.95<br>479.50<br>577.62 | 188.50<br>286.75<br>156.60<br>——<br>——<br>—— | 582.90 | 339.30 | —— | 11.10 | —— | —— | 265.0 | 94.0 | 250.00 | 250.00 |
| Chloride | 676.05<br>688.70<br>—— | 667.19<br>578.65<br>738.40 | 517.70<br>—— | ——<br>1,072.08 | 646.10<br>575.10<br>——<br>532.50<br>507.65<br>619.00 | 447.00<br>571.55<br>472.15<br>——<br>——<br>—— | 628.35<br>—— | ——<br>1100.50 | —— | 7.00 | —— | —— | 415.0 | 146.0 | 375.00 | 500.00 |
| Potassium | 64.66 | 15.82 | —— | —— | 25.82<br>26.07 | 10.22<br>13.67 | 97.04* | 11.72 | —— | 7.46 | —— | —— | —— | —— | 20.00 | 137.00 |
| Calcium | 14.04 | 26.47 | —— | —— | 10.45<br>8.60<br>9.10 | 60.00<br>42.08<br>—— | 15.68 | 54.70 | —— | 1.48 | —— | —— | —— | —— | 10.00 | 12.00 |
| Magnesium | 23.54 | 253.76 | —— | 176.60 | 11.09<br>9.80<br>7.31<br>8.01 | 179.07<br>186.20<br>171.67<br>—— | 6.83* | 179.23 | —— | 0.32 | —— | —— | —— | —— | 2.50 | 12.00 |
| Phosphorus | 14.49 | 10.69 | —— | —— | 25.17<br>28.43<br>20.40 | 5.38<br>6.00<br>—— | 9.73 | 6.65 | —— | 7.25 | —— | —— | 4.0 | 36.0 | 9.00 | 118.00 |

* Turtle urine (Lewis). Analyses of the urine from 2 turtles show certain differences as follows: non-protein N, 62.0; urea 60.0; uric acid, 36.0; $NH_4$, 13.4; creatine, 18.6; creatinine, 2.7; urea N 28.0; uric acid N, 12.0; $NH_4$ N, 11.0; creatine N, 6.0; creatinine N, 1.0; undetermined N, 4.

† A more complete analysis of fowl's urine available from data given by Davis (1927) and Sharpe (1912) is placed here since no corresponding analysis of plasma was given by them. Non-protein N, 100.0; urea, 22.3; uric acid, 187.7; $NH_4$, 21.0; creatinine, 21.5; urea N, 10.4; uric acid N, 62.9; $NH_4$ N, 17.3; creatine and creatine N, 8.0; undetermined N, 1.4; Na, 418.78; Cl, 486.50; 199.20; Ca, 281.59; P, 41.80.

Analysis of table 2. 2. Concentration of urinary constituents by the kidney.

| CONSTITUENT | X CONCENTRATED | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sygnathus | Hippo-campus | Lophius | Muraena | Frog | Fowl | Man |
| Non-protein nitrogen | 1.5–2 | 1.0 | 3.0 | 0.94 | 1.2 | | 36.0 |
| Urea | | 0.93 | 0.6 | 1.0 | 1.1 | | 69.0 |
| Uric acid | | | | | 0.13 | 32.8 | 13.0 |
| Ammonia | | | 7.0 | 4.0 | | | 60.0 |
| Creatine | | | | 8.0 | | 2.8 | |
| Creatinine | | | 29.0 | 30.0 | | 3.0 | 72.0 |
| Sodium | 0.37 | 1.0 | 0.3–0.6 | 0.59 | | 0.35 | 1.0 |
| Chloride | 0.8–1.0 | 2.0 | 0.7–1.0 | 1.5 | | 0.35 | 1.3 |
| Potassium | 0.24 | | 0.4 | 0.12 | | | 7.0 |
| Calcium | 2.0 | | 6.0 | 3.5 | | | 1.2 |
| Magnesium | 11.0 | | 16.0 | 26.0 | | | 4.0 |
| Phosphorus | 0.74 | | 0.2 | 0.68 | | 9.0 | 13.0 |

DISCUSSION. These data as presented make clear the initial scope of the study and constitute an anatomical and physiological basis of comparison of the blood and urine of the various fish studied. Similar data for the frog, turtle, fowl and man have been made conveniently available. The distinction between meso- and metanephros becomes largely embryological as a result of these studies and the reservation (Cushny, 1926, p. 84) with regard to the mesonephros of the lamprey which he says "may differ entirely in its functional activity from true renal epithelium" is probably not true for the lamprey or for the fish presented here. It is obvious from figures 1 to 4 that a definite modification of the renal tubule occurs in association with its glomerular attachment. The important features of this modification appear to have arisen simultaneously with the development of the glomerular-connected tubule, the nature of which is shown in figure 3.

The aglomerular tubules described here as well as by Verne (1922) show, as regards the ciliary and brush-border development, that the cells of such tubules contain cilia on their inner border beginning at the apex where they are longest and continuing through the apical third approximately, where they grade into the brush-border aspect. With the development of the glomerular tubule, a constriction occurs near the point of transition thus differentiating the two convolutions, transections of which are shown in figure 3. It is probable that this first, ciliated convolution becomes reduced in the frog and turtle and is represented by the neck segment while the brush-border segment becomes the proximal convolution proper of the kidney of higher vertebrates. This does not account for the transposition of the ciliated intermediate segment of frog and turtle although this segment occurs in the same relative position in the tubule.

The consistent presence in the tubule of the aglomerular kidney of two segments which are the approximate equivalents of the proximal and distal convolutions of the glomerular tubule in the kidney of higher vertebrates, one with, the other without a brush border, together with other data available but not presented in this connection, does not justify the functional comparison of the brush-bordered type of renal epithelium with that of intestinal epithelium.

The progressive differentiation in the visceral layer of the capsular epithelium may not be regarded as without significance. According to Huber (1928, p. 687) "In fully developed renal corpuscles of mammalia and especially of man it is often difficult to make out clearly a continuous epithelial layer. . . . . The epithelial layer has been regarded as consisting of a thin nucleated, syncytial layer of protoplasm or as disappearing entirely as a continuous epithelial layer in the fully developed renal corpuscles of mammalia." This may mean that the thicker visceral layer of the capillary tuft in fish, frog and turtle modifies in a way simulating secretion the "provisional capsular urine" of amphibia, certain constituents of which have been analyzed by Wearn and Richards (1925) and by Schmitt and White (1928); whereas a process more nearly resembling filtration may occur in mammals.

Cushny's statement (p. 6) that "whether the tubules (mammalian) are capable of secretion or not, they cannot be the exclusive route of elimination of any constituent" seems somewhat dogmatic. It is difficult to find in the anatomy or physiology of the aglomerular kidney or in that of the kidney of higher vertebrates the basis for conceiving that the attachment of a capillary tuft to a tubule induces a reversal of the secretory process (selective reabsorption as held by Cushny). Moreover, Verne presents evidence that in the aglomerular tubule of Lophobranchs, of which he made a careful physiological study, one tubule may be active while another is not. Therefore, even here, the aglomerular tubule anticipates the glomerular in the alternation of the glomerular activity of the latter (Hayman and Starr, 1925).

It seems unlikely that the epithelium of the aglomerular tubule acts like a filter and while the vertebrate kidney is not a gland considered as manufacturer, urine also is not plasma. The hypotonic urine of the aglomerular kidney is significant in this connection as well as concerning the secretion of water. The functional difference in the segments or the aglomerular tubule is not clear either as a result of these studies or those of Verne, but the indications given by Turchini (1922), Verne, and by these studies leave little doubt as to the locus of transfer of dyes, i.e., at least through the first segment or equivalent of the proximal convolution which constitutes the major portion of the aglomerular tubule.

The hypotonic urine of frog, turtle and fowl may similarly as in that of

the aglomerular fish be the result of a secretory process with or without "selective reabsorption," or with relatively less of the latter than in the kidney of mammals. Consideration is thus given to progressive modification in the tubule proper, in the visceral layer of the capsule and to the consistent presence of a thin segment of variable length in the loop of Henle in mammals. The site of reabsorption, if such occurs in the aglomerular tubule, is probably in the equivalent of the distal convolution. That reabsorption occurs in the distal convolution of the frog's mesonephros is indicated by the studies of Bensley and Steen (1928). In the mammal it occurs probably in this convolution and also in the thin segment of the loop of Henle.

The functional difference in the concentration of urinary constituents productive of hypo- and hypertonic urines seems to point anatomically to the warm-blooded, avian kidney where reptilian, variously intermediate and mammalian types of tubules occur. The hypotonic urine of the fowl may be thus the result of the greater, combined activity of the first two types. But the concentration of urinary constituents "through vital absorption in the epithelium of the tubule," or the elaboration of urine "by reabsorption through vital cellular activity" (Cushny, p. 125) may be less "occult" (Cushny, p. 60) than the conception of Heidenhain (1874), but the distinction leaves much to be desired.

The casual development of glomeruli associated with greater differentiation of the relatively more original tubular pattern, the initial development in fish of an aglomerular mesonephros followed according to Audigé (1910) by that of glomeruli at the onset of sexual maturity, the alternation in tubular activity, the fact that the aglomerular kidney of Lophius may excrete 3 times as much urine per day as the glomerular one of Muraena, the response to dyes and diuretics by the aglomerular kidney (Verne, l.c.), the hypotonic urines of the lower vertebrates together with the phenomenon (?) of renal compensatory hypertrophy seen in man and experimentally induced in other mammals, all seem to indicate a rôle for the tubule not precisely accounted for by the filtration-reabsorption hypothesis.

## SUMMARY

1. The aglomerular mesonephros of three genera of bony fish has been studied physiologically and compared variously with a fourth having both aglomerular and glomerular tubules.

2. The ready excretion of dyes through the aglomerular kidney, visible during excretion in the apical two-thirds or more of the tubule (which is ciliated to brush-bordered) lends support to the microscopic observations of the living kidney made by the senior author to the effect that readily excretable, non-toxic dyes when given in proper amounts, are excreted

chiefly through the epithelial cells of the proximal convolution in the renal tubule of the rat and in amphibia, and normally are not reabsorbed from the lumen of the tubule. It does not follow because dyes are thus not reabsorbed from the lumen of the tubule, that water or certain urinary constituents may not be reabsorbed.

3. A urine of similar composition is excreted through the aglomerular and partly glomerular kidney. The comparison between the composition and concentration of the constituents of the urines of the fish as well as similar data assembled for the frog, turtle, fowl and man is conveniently made possible in the table containing the analyses, and their simplification as given in the text.

4. The venous blood supply and not tubule inadequacy as conditioning the relative amounts of urine excreted per day by these fish is suggested.

5. The functional inferiority of the aglomerular or partly glomerular mesonephros of fish when compared with that of the frog is not obvious from these studies. The mesonephric portal system in connection with tubular secretion (secretion as defined for the kidney) constitutes the probable basis for the differences in the urines excreted by such mesonephroi as compared with metanephroi.

6. The aglomerular kidney constitutes a natural preparation for the study of tubular activity in its more primitive state. The subsequent differentiation of the renal epithelium observable in the kidney of higher vertebrates when considered in connection with its functional performance, does not predispose to a belief that this activity as it must occur in the aglomerular and venous kidney has been significantly perverted in the glomerular kidney.

7. The proximal convolution of the tubule of higher vertebrates as well as its prototype in the fish kidney is the longer and more differentiated segment. It appears from direct observations of the activity of its epithelium to be equally or more important than other segments. Whatever its function, it seems clear that it is normally not reabsorptive.

8. Filtration pressure, so largely discussed with reference to phases of activity dealt with in the Ludwig-Cushny hypothesis, is hardly relevant to a discussion of the activity of aglomerular and *venous* kidneys.

9. The careful preservation of a solely venous blood supply to the tubules of the mammalian kidney (by the mediation of glomeruli) may be significant in the induction of probable permeability changes requisite for the passage through the renal epithelium of urinary constituents.

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# INITIAL AND RECOVERY HEAT PRODUCTION IN SMOOTH MUSCLE BASED UPON EXPERIMENTS ON A URINARY BLADDER-NERVE PREPARATION FROM TERRAPIN

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Received for publication July 7, 1928

In earlier work (1914) it was observed that the urinary bladder of terrapin (Malaclemmys palustris) when isolated and emptied of its contents becomes a suitable preparation for the study of heat production in smooth muscle.

When empty this organ falls naturally into two elongated narrow bags that lie in contact with one another. The two bags, or lobes, depending from their common base, the "neck" of the bladder, may be set astride the warm junctions of a flat coil thermopile quite in the manner that a pair of frog's sartorius muscles (depending from their point of common attachment) may be set astride a flat-coil thermopile. If the tips of the lobes are then tied together and fastened to a fixed point, and a suitable support inserted at the crotch, where the lobes join the neck, upon applying gentle traction the elongated lobes are stretched still longer. This effect, due to the great plasticity of the tissues of this organ, tends to pull transverse and diagonal fibers around to lie alongside the naturally parallel fibers of the muscular walls. No smooth muscle organ probably has less intermixture of other tissues. We thus have a preparation of almost pure smooth muscle fibers most of which lie parallel to one another, presumably none of which have suffered trauma. If the lobes are considerably longer than the rows of warm junctions on the thermopile (and the lobes of our specimens are often much longer) then the thermopile may be inserted between them in a region where their cross-sections are most uniform. In this case the method of direct calibration of thermopile performance can be made use of. Preliminary experiments on our preparation with the direct method of stimulation and the direct method of thermopile calibration, however, have failed so far to yield results at all comparable in uniformity with those obtained by the indirect method of thermometry and from automatically contracting muscle, or muscle responding to electrical stimulation of its nerves. In the measurement of heat production the desirability of stimulating muscles through their nerves, in case automacity is absent, has already been dwelt upon in other papers from this laboratory (1922, 1925, 1927).



Aided by a grant from the Henry Strong Denison Medical Foundation

In the present paper we are reporting only the results obtained when the organ had been stimulated to contraction through its motor nerves, or had exhibited simple spontaneous contractions. The analyses of the thermomyograms so obtained have been made by the method of indirect thermometry, that is, calculating temperature changes from the theoretical performances of the thermopile.

The terrapin bladder often exhibits automatic contractions but these may be of a frequency too rapid for the best performance of thermopile and galvanometer, and they are apt to consist of twitchings of isolated fiber-bundles rather than a simultaneous contraction of the whole muscle mass. The isolation of the motor nerves to the urinary bladder of the terrapin, therefore, became a highly desirable addition to the technique of this investigation. One of us (F. W. L)., therefore, set about finding the nerves, and perfecting a procedure of isolating them with the bladder intact. And since these nerves have not yet been described in the literature, so far as we have learned, a rather detailed description of them is here given.

In most specimens of terrapin there are two pairs of motor nerves to the bladder. The smaller of these, which is sometimes absent, but which when present carries a large part of the motor fibers to the bladder, comes from the anterior root of the twelfth dorsal nerve. This root also gives fibers to the sciatic nerve. But the branch supplying the bladder (the minor vesical nerve) although closely bound to the sciatic for a short distance, maintains its identity throughout and leaves the sciatic trunk to pass ventrally between the ilium and the second glutaeus muscle. (Names of muscles are based upon the terminology in Owen's *Anatomy of Vertebrates*). On reaching the ventral surface of this muscle, the minor vesical nerve crosses it, thus reaching the cloaca, as shown on the (reader's) right side of the drawing (fig. 1). Often a small branch is given to the wall of the anterior part of the cloaca.

The larger, or major vesical nerve, is a continuation of the anterior root of the thirteenth dorsal nerve. Just after emerging from the spinal column this nerve gives off a long branch which passes superiorly around the cloaca to the rectum and adjoining structures. The main nerve pierces the second glutaeus muscle and proceeds ventrally in close apposition to the wall of the cloaca, between it and the flexor caudae lateralis muscle. In this part of its course the nerve supplies one or more branches to the wall of the middle part of the cloaca, but finally comes to lie beside the minor vesical nerve, as shown in the figure.

The two vesical nerves run side by side from this point, but do not anastomose, at least not before entering the bladder. They cross the neck of the accessory bladder, and continue along the lateral wall of the cloaca, passing dorsally the cloacal ends of the oviducts. Just before reaching the neck of the main bladder, they pierce the cloacal wall and continue their

course to the bladder beneath the surface. In this study they have not been followed beyond the neck of the bladder by any anatomical method.

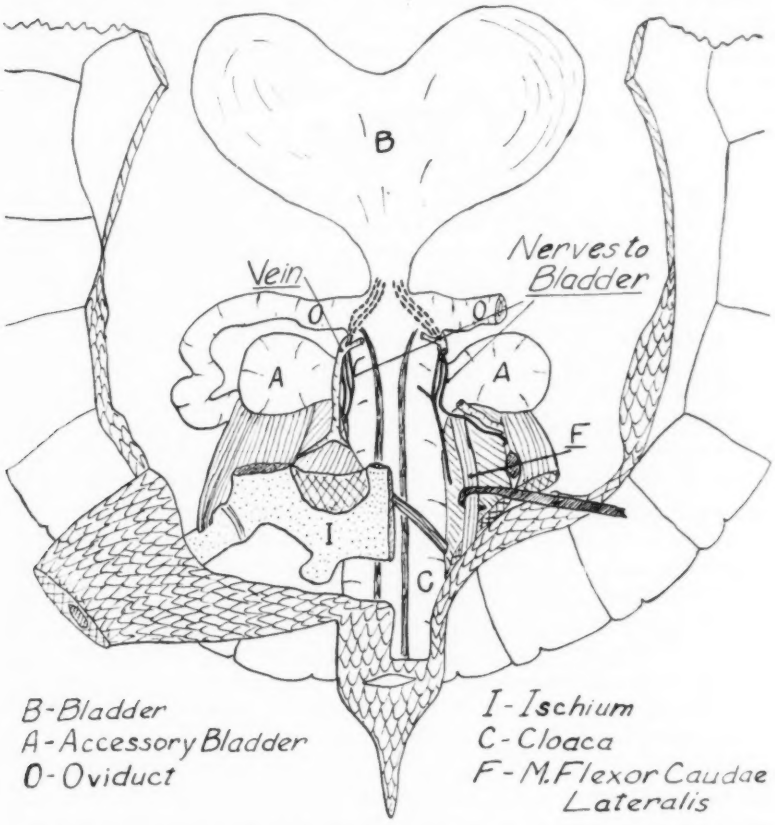


Fig. 1. A free-hand drawing, largely diagrammatic, of posterior body-cavity of terrapin carcass with plastron removed; ventral view showing urinary bladder partly filled with urine, and the region of dissection preparatory to the isolation of the organ with its motor nerves attached. Compare with description in text. Note the two pairs of "nerves to bladder" running forward alongside the cloaca; their continuations are shown in broken lines to indicate that they pass along on the dorsal aspect of the caphalad end of the cloaca to enter the walls of the "neck" of the bladder as many fine filaments.

When the minor vesical nerve is absent, the major nerve is considered to carry all the motor fibers to the bladder.

This study of the nerve supply of the bladder was secondary to the main

problem of its heat production. Stimulation of the nerves just described always caused the bladder to contract, but we have not demonstrated that there are absolutely no other motor tracts to this organ. It was found that stimulation of either nerve on either side caused both lobes of the bladder to contract. Such tests were made on only a few preparations, but always gave the result stated. In all the heat production experiments all the nerves were stimulated simultaneously.

In making a urinary bladder-nerve preparation the following precedure was carried out. After crushing the brain the animal was fastened on its back, the plastron removed, and the animal bled by cutting the aorta in order to ensure a bloodless field for rapid dissection. The entire pelvic girdle was removed (after cutting all muscles connecting it with the legs and body) as well as the femora and ilia. Connective tissue and fat were cleaned away until the cloaca and communicating organs were laid bare. The nerves can be picked up most easily as they cross the neck of the accessory bladder. They can be located readily since they lie just beside or beneath a prominent vein, as depicted in the drawing (fig. 1). Once found, the nerves were dissected free and followed in both central and peripheral directions. The major nerve can be traced best if the flexor caudae lateralis muscle is pulled away from the cloaca, as in the drawing. Both nerves were sectioned near their spinal origins. Peripherally they were dissected out nearly to the point beneath the oviducts (most specimens were female) where they pierce the cloacal wall. The oviducts, ureters and rectum were then cut away as completely as possible. If the sectioning of these structures, however close to the cloaca, is performed carefully, there is no danger of injuring the nerves. Finally the cloaca was transected just below the point at which the dissection of the nerves was suspended. The preparation thus obtained bore very little tissue other than that actually desired, —the main urinary bladder and its nerves.

Method of experimental procedure. The instrumentarium, thermopiles, galvanometer, muscle chamber, isometeric or tension lever, and graphic registration were all similar to those used and described in previous papers on heat-production from this laboratory, first in 1914, and especially enlarged upon in the papers of 1922, 1925, 1927, and 1928. The thermopile used was a modification of the original wicket-gate (Gittersäule) type of Bürker, later amplified by A. V. Hill and called by him the flat coil thermopile. The extreme slowness of the contraction of this smooth muscle preparation enabled us not only to use the d'Arsonval low-resistance galvanometer (whole period, 24 seconds) for recording the thermoelectric effects, but also to work with the muscle chamber at room temperature. This enabled us also to use the smoked-drum (revolving at a rate of about 1 mm. per second) for graphic registration instead of the more troublesome

and, for our purposes, no more accurate photographic method. The writing tips of the recording lever made vertical excursions on the drum-surface; their spatial relations along a vertical was put in the record by frequent "scratch-marks." Frequent tests of galvanometer freedom and sensitivity were made throughout an experiment by switching over to the standard calibration circuit. Working at room temperature not only obviated long waits for temperature equilibrium, but also reduced the stratified differentials of muscle-chamber temperatures so much that correction for the minute vertical displacement of thermopile coil during a muscle contraction was found to be no longer necessary; the basal temperature level of resting muscle also kept nearer the galvanometer null point for longer periods of time.

The muscle was studied when surviving *a*, in moist atmospheric air, indicated in protocols as 21 per cent oxygen; *b*, in moist 99 per cent oxygen, after displacing completely the previous gaseous medium first with Ringer-$O_2$ then displacing the Ringer-$O_2$ with the 99 per cent $O_2$, and *c*, in a completely anoxybiotic condition. The method of introducing Ringer's fluid and gases into the muscle chamber and rendering the chamber practically air-tight during an experiment was the same as that described for the study of anoxybiotic heat production of the beating heart (Snyder, 1928).

To obtain a condition of complete anoxybiosis the air in the muscle chamber was displaced first completely with Ringer-$N_2$ solution. All the $N_2$-gas used in this investigation was gotten by passing the commercial $N_2$-gas, containing about one per cent oxygen, through two pyrogallol wash-bottles connected in tandem. Therefore, when we use the terms "nitrogen," "pure nitrogen" and "$N_2$-gas" in this paper we mean nitrogen so purified. We considered the final gas to contain at most no more than 0.1 of one per cent oxygen. But in order to make more useless free-$O_2$ that possibly was held back within the tissues we used cyanide, that is Ringer + $N_2$ + m/3000 NaCN. The pH of this cyanide solution was brought back to equal that of the plain Ringer by the addition of equivalent amounts of n/10 HCl, special precautions being taken by the method (described for the heart muscle study, 1928) to prevent gaseous exchanges between the inside and outside of the containers. All the apparatus, solutions, gases and materials used were kept in the experiment room for many hours preceding the experiment to give them time to take on the temperature of the room. After filling the muscle chamber with nitrogen as described above, this in turn was displaced completely with Ringer-$N_2$-CN solution. The cyanide was allowed to act on the surviving preparations from 7 to 15 minutes and then displaced with $N_2$-gas.

In any case after effecting the desired medium for the muscle, and when temperature conditions were near enough equilibrium we proceeded to stimulate the nerves with the tetanizing current $\frac{3}{4}$ to 3 seconds to call forth mechanical responses, unless, as sometimes happened, the muscle showed satisfactory automatic contractions. One great advantage of stimulating

smooth muscle through its nerve is that the maximal strength of current necessary to evoke maximal contractions is exceedingly weak, the secondary often being 12 cm. distant from the primary (Harvard coil), and always with but one dry cell in the primary. Tests showed that the heat produced by this feeble current passing through electrodes and short stretch of nerves had no effect on the thermopile circuit. Had we determined the chronaxie of the preparation to a direct current we may have been able to use a still weaker current.

TREATMENT OF RESULTS. The graphic record shown in figure 2 is here reproduced because it well illustrates the extremes of "good" and "bad" behavior that our preparations sometimes exhibited after they had been isolated, harnessed and mounted in what we thought to be faultless fashion. It is, therefore, worth while to describe this record in some detail.

The "upper lap" of the record consists of the usual three trace-lines, T being that of the combined time-stimulation signal lever, M being that of the tension lever (provided with Ludwig vertical writing tip, down-stroke representing rising tension), and $\theta$ that of the galvanometer-spot recorder (also provided with vertical writing device) upstroke of which indicates rise, downstroke fall of temperature of the muscle. It will be noted that with the tension rise, shown by M, there occurs simultaneously a sharp temperature rise in $\theta$, a rise that is promptly converted into a fall of equal rate during early relaxation; a marked second, but more prolonged, rise of temperature appears during the later and much prolonged part of the muscle relaxation. That these two temperature rises represent two major phases of heat production is as obvious as the rise and fall of the mechanical tension itself. The same sequence of events is repeated as responses to the second stimulation of the muscle. The stimulus in both cases was a tetanizing induced current applied for three seconds to the nerves supplying the organ, the coil being a Harvard instrument with secondary at 9 cm. distant and 1 dry-cell in the primary circuit. The muscle-nerve preparation had been resting in moist atmospheric air at 19.5°C. for $2\frac{3}{4}$ hours. The "resting heat production" or zero level of muscle temperature was near the galvanometer null point. This is indicated in the record by the low rate of drift of the thermomyogram base-line, a line that has been arbitrarily added to the record.

The wave of temperature rise during the muscle contraction is taken to be a simple wave and to indicate a sharp, short-lasting evolution of heat; the wave of temperature rise during the muscle relaxation is taken to be a compound wave of at least three simple waves, each subsiding to base-line nearly simultaneously as its successor reaches its highest point. The measure of value for the wave during contraction thus becomes simply its vertical height, that of the compound wave during relaxation, the sum of the vertical height of the three constituent simple waves.

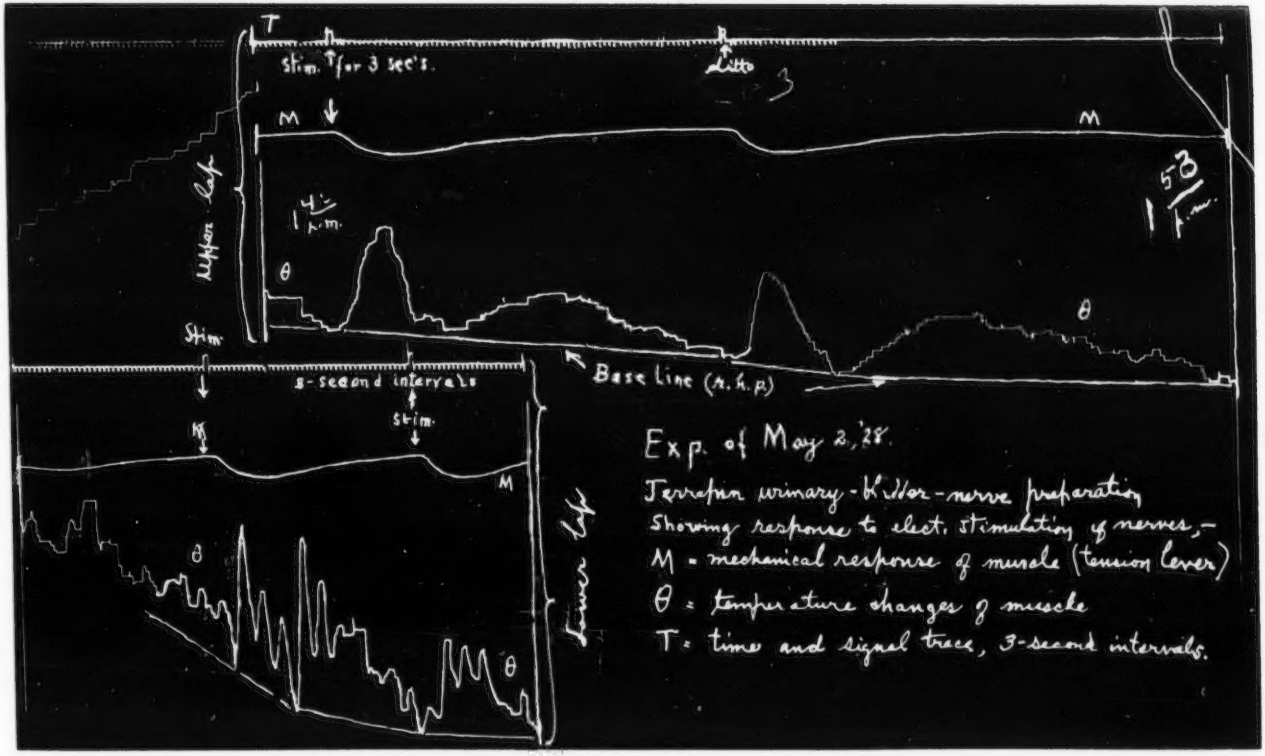


Fig. 2. Showing thermomyograms of the urinary bladder of the terrapin in response to faradic stimulation of its motor nerves. Reduced to $\frac{2}{3}$ of the original size. The trace-lines for the most part have been retouched for purposes of photographic reproduction. The figure shows distinct "initial" and "recovery" temperature rises for two separate contractions (see "upper lap").

The data in the graphic record together with that in the experimental notes enable us to make the necessary analyses of the results which are as follows:

| MYOGRAM NUMBER | MAXIMAL TENSION | TEMPERATURE RISES | | HEAT PRODUCED, IN CALORIES $\times 10^5$ | | |
|---|---|---|---|---|---|---|
| | | Initial | Delayed | $\frac{H_i}{T}$ | $\frac{H_r}{T}$ | $\frac{H_t}{T}$ |
| | *gm.* | *mm.* | *mm.* | | | |
| 1 | 37.5 | 24 | 26 | 3.52 | 3.82 | 7.34 |
| 2 | 38.9 | 21 | 28 | 2.97 | 3.96 | 6.93 |

This tells us that during the whole cycle of each muscle response a total of about 71 micro-calories of heat was produced per gram muscle per gram maximal tension exerted, less than half of which was developed in one stage during the contraction period (lasting about 30 seconds), the rest, the "delayed," during the period of relaxation (lasting about 4 minutes).

In the experiments about to be reported the initial and delayed waves in the thermomyograms are often not so sharply separated, on the other hand the simple waves making up the compound delayed wave are often much better separated than they are in the thermomyograms of figure 2. Again they sometimes show much deviation even when conditions were thought to have been identical. One kind of deviation from the type we have just described is well illustrated by this same muscle and in the same record reproduced in figure 2. In the part of the record marked "lower lap" the thermomyogram ($\theta$) shows a number of oscillations that have nothing in common with the accompanying tension changes in M. Such oscillations may presage an impending break in the thermopile coil (oscillating resistance, or an open, undamped galvanometer circuit); but in this particular case the thermopile was in perfect condition. The oscillations here we believe are due to a condition of idiomuscular twitching, or a sort of group fibrillation, that we observe now and then to come upon our preparations. Sometimes these automatic twitchings are spaced far enough apart and occur well enough in unison to be communicated to the muscle lever, when they appear in the myogram tracings as minute spontaneous contractions of more or less regular rhythm, but of a frequency too high to permit the galvanometer (and thermopile) to follow them in anything more than a fitful, imperfect way,—as is probably the case in this "lower lap" of figure 2. Only here the myogram (M) fails to show any sign of the twitchings, whence we infer they are too irregular and antagonistic to exert a measurable pull on the muscle lever. But in spite of these "idiomuscular" oscillations of the thermomyogram one notes that the longer and slower initial and recovery waves of heat production are still to be made out when the whole muscle contracts in response to stimulation of its nerves. The more frequent oscillations are simply superposed upon the slower and longer waves, analysis of which shows values equal to those found above for the less complicated thermomyograms of the "upper lap" of the record. It need hardly be added that no records complicated with marked fibrillation or idiomuscular twitchings were used in the results submitted below. To our gratification we soon found that our preparations could be freed of these irregular twitchings by completely withdrawing oxygen and

treating with cyanide; however, now and then return to an atmosphere of high oxygen tension precipitated a return of the twitchings.

In a few experiments, one after a long series of observations, the results had to be discarded because some essential in the technique had been overlooked, or some accident occurred to the preparation, for example a partial dislodgment of the thermopile, which was only discovered at the end of the experiment. But a number of experiments came through without mishap and these form the basis of this paper.

The method we have adopted for measuring the whole of the delayed heat, we realize is open to objection. It would be more satisfactory had we checked it with the more direct method that Hill and Hartree (1920) proposed and applied to skeletal muscle; work to that end is in progress at present to do this with a smooth muscle preparation more suitable for the purpose. In this paper, however, we have had to content ourselves with the more indirect method that has been used heretofore in this laboratory. Among the reasons we have for adopting our method when working with the irregular shaped, involuntary organs, is that of the slowness of all the processes of contraction, which is more pronounced in smooth muscle, contraction of smooth muscle being still slower at room temperature than terrapin heart-muscle at 0°C. This prolongation of the recovery period, as was observed also for the ventricle muscle (1922), permits the recording apparatus to follow the several outbursts of delayed heat as distinct waves in many of the thermomyograms of urinary bladder. Comparing thermomyograms made up of these discrete waves with those made up of the more fused type (as shown in the delayed heat waves in fig. 2) enables one to make fairly reliable judgments as to the real slope of the descending limbs of the simple waves that make up a compound wave. The slopes, and thus their relative effects on ascending slopes, having been determined simply by curve fitting, the vertical heights of the simple waves summed represent the total delayed heat.

The initial wave usually stands out sharp and clear and its analysis is a simple task. This cannot be said always of the compound recovery wave; although often enough its constituent simple waves stand out with great clearness and beauty, and the task of analysis is sure and swift. But all too often the last one or two of these constituent waves are small and doubtful, or in case of automaticity the initial wave of a new contraction is superimposed. All doubtful thermomyograms that could not be analyzed with certainty had to be discarded. This required more and longer experiments than if all the records were perfect, but has rewarded us finally with a fair abundance of material and the assurance that further experiments will not alter the character of our results.

Results. In presenting our results we must be content with a complete tabular statement of the measurements of all the thermomyograms, and their corresponding tension curves, belonging to one experiment only.

TABLE I

*Experiment of May 23, 1928*

Terrapin urinary bladder-nerve preparation, contracting mass 2.29 grams. Dead weight on lever, 6.11 grams. Temperature, muscle chamber, 22.3°C. All myograms evoked by faradic stimulation of nerves. 1 mm. deflection of galvanometer = $3.24 \times 10^{-5}$°C. 1 mm. excursion m. lever = 3.51 grams.

$$\frac{H}{T} = \frac{\theta \text{ in mm.} \times 3.24 \cdot 10^{-5} \times 0.825 \times 2.29}{(\tau \text{ in mm.} \times 3.51) - 6.11}$$

| TREATMENT OF MUSCLE-NERVE PREPARATION | TIME | | NUMBER OF MYOGRAM | STIMULATING CURRENT | | MAXIMUM TENSION ($\tau$) | GALVANOMETER DEFLECTION | |
|---|---|---|---|---|---|---|---|---|
| | | | | S. C. | Time | | Initial $\theta_i$ | Delayed $\theta_r$ |
| | *hour* | *minute* | | *cm.* | *seconds* | *mm.* | *mm.* | *mm.* |
| I. After resting 2 hours, and continuing in 21 per cent *oxygen*..... | 1 | 20 | 1 | 12 | 1.5 | 13.4 | 26 | 27 |
| | | | 2 | 12 | 1.5 | 14.5 | 20 | 16 |
| | | | (3) | 12 | 1.5 | 15.5 | 19 | 29 |
| | | | 4 | 12 | 1.5 | 14.0 | | * |
| | | | 5 | 12 | 4.5 | 15.5 | 21 | 22 |
| | | | 6 | 12 | $2\frac{1}{4}$ | 13.2 | 15 | 25 |
| | | | 7 | 11 | $2\frac{1}{4}$ | 15.0 | 16 | 30 |
| | | | (8) | 11 | 1.5 | 13.5 | | |
| | 1 | 40 | 9 | 11 | $\frac{3}{4}$ | 13.1 | 16 | 29 |
| II. After *CN*-bath, resting 40 minutes in $N_2$ and continuing in $N_2$-gas... | 2 | 30 | (10) | 12 | 1.5 | 11.5 | | 4 |
| | | | 11 | 12 | 3.0 | 11.0 | 5 | 3 |
| | | | 12 | 11 | 3.0 | 11.0 | 10 | 4 |
| | | | 13 | 11 | 3.0 | 11.0 | 11 | 5 |
| | | | 14 | 11 | 1.5 | 10.2 | 10 | 6 |
| | | | 15 | 11 | $\frac{3}{4}$ | 8.8 | 3 | 5 |
| | | | 16 | 11 | 3.0 | 10.3 | 7 | 4 |
| | | | 17 | 11 | 3.0 | 10.0 | 9 | 7 |
| | | | 18 | 11 | 1.5 | 9.3 | 8 | 4 |
| | 3 | 20 | 19 | 11 | $\frac{3}{4}$ | 8.0 | 6 | 5 |
| III. After resting in 99 per cent *oxygen* 20 minutes, then continuing in 99 per cent *oxygen* | 4 | 05 | 20 | 11 | 3.0 | 13.7 | 16 | 34 |
| | | | 21 | 11 | 3.0 | 13.8 | 19 | 31 |
| | | | 22 | 11 | 3.0 | 14.0 | 21 | 27 |
| | | | 23 | 11 | 1.5 | 13.3 | 20 | 28 |
| | | | 24 | 11 | 1.5 | 14.0 | 20 | 15 |
| | | | 25 | 11 | 1.5 | 14.2 | 11 | 23 |
| | | | 26 | 11 | 3.0 | 14.3 | 29 | 21 |
| | | | 27 | 11 | $\frac{3}{4}$ | 12.3 | 14 | 19 |
| | | | 28 | 11 | 3.0 | 14.6 | 31 | 45 |
| | | | 29 | 11 | 1.5 | 13.9 | 23 | 28 |
| | | | 30 | 11 | 1.5 | 14.4 | 35 | 19 |
| | | | 31 | 11 | 3.0 | 13.7 | 22 | 29 |
| | | | 32 | 11 | 1.5 | 13.0 | 25 | 33 |
| | 5 | 25 | 33 | 11 | 3.0 | 14.4 | 24 | 39 |

* The blank indicates that that part of the thermomyogram failed to be recorded.

This will be followed by a tabular summary of the mean results of analyses based upon similar measurements of our successful experiments, and finally with what general conclusions they seem to warrant.

By presenting all the data for one experiment as we do in table 1 the reader may see the time covered by an experiment, the variations in relative strength and duration of the stimulus applied to the nerves, the variations in the maximal tension evoked by these stimulations, as well as the variations in their thermal responses, both during the initial and recovery stages of each contraction. Other data pertinent to the calculations and leading up finally to absolute values appear in the legend to the table.

For more detail as to the method of, and the object gained by these calculations the reader is referred to the papers by others as well as those from this laboratory. One consideration not heretofore emphasized enough may here be expanded. All organs are made up of a mixture of tissues; a muscle-organ when contracting produces heat in the separate fibers and the final rise of temperature of the organ depends on an equal distribution of the heat among all the non-contracting elements lying between the muscle fibers, and covering the surfaces of the organ, as well as the muscle fibers themselves. The relative weights, to say nothing of absolute weights, of the contracting and non-contracting, of the heat-producing and non-heat-producing masses making up the organ, cannot be known in any given case. But fortunately this knowledge is not required to determine the total heat appearing in the organ, providing that the total weight of the organ warmed and the actual rise of temperature of the organ are both known. This is even true when only a portion of the total muscle fibers of an organ contracts. All this follows because in the algebraic equation expressing the ratio, $\frac{H}{T}$, that is the heat produced by one gram of muscle per one gram of the maximal, total tension developed by an organ, the weight of pure muscle fibers (that is only the weight of contractile elements producing the heat and the tension in any particular act of contraction) appears in both the numerator and the denominator and, therefore, cancels out of the equation entirely. Therefore, the absolute weight of the contracting fibers need never be known. (For the development of the equation see Snyder, 1927, p. 735.)

It should be stated that the term "dead weight" that appears in the denominator of the equation, given in the legend to table 1, is the total weight of muscle-nerve preparation plus a hard rubber ring holding the muscle between the lobes of the bladder, plus the metal cable connecting the latter with the muscle lever. This weight depresses the muscle lever from its zero tension-mark on the drum-record and must, therefore, always be subtracted from the total weight represented by the extreme excursion of the myogram. The value placed under T is tension thus corrected.

It will be observed that the muscle tensions become somewhat less after the muscle has been treated with cyanide and kept in an $O_2$-free atmosphere. The depression of the waves of temperature ($\theta_i$ and $\theta_r$), however, is still greater. Upon reducing these values to standard conditions (calories of heat per gram muscle per gram total tension exerted) that is, to H/T ratios, we see that the initial heat also has been reduced (see table 3 for the absolute values). But the delayed heat has been reduced even still more. From this we see that in the absence of free oxygen, and, presumably, aided by the cyanide treatment, during the cessation of all oxidations, the amount of heat per unit of tension exerted is much less.

Passing now to the third section of table 1 we find that upon washing

TABLE 2

*Summary of ranges of total tensions and $H_1/T$ ratios in calories $\times 10^5$*

| TREATMENT OF PREPARATIONS | DATE OF EXPERIMENTS 1928 | NUMBER OF THERMOMYOGRAMS | TENSIONS | | TOTAL HEAT PRODUCTION PER GRAM MUSCLE AND PER GRAM TENSION | |
|---|---|---|---|---|---|---|
| | | | Minimum | Maximum | Minimum | Maximum |
| | | | *grams* | *grams* | | |
| I. *Oxygen*, 21 per cent | iv/11 | 5 | 22.9 | 41.3 | 2.93 | 4.68 |
| | iv/18 | 14 | 28.3 | 38.0 | 2.16 | 6.49 |
| | iv/2 | 2 | 37.5 | 38.9 | 6.97 | 7.58 |
| | v/23 | 7 | 39.9 | 49.4 | 5.00 | 7.93 |
| II. *Cyanide*, then pure $N_2$-gas | v/16 | 8 | 50.7 | 80.0 | 3.01 | 5.24 |
| | v/18 | 5 | 39.5 | 58.2 | 1.95 | 2.60 |
| | v/23 | 9 | 22.0 | 34.3 | 1.41 | 3.38 |
| | vi/6 | 6 | 11.3 | 14.5 | 3.90 | 6.35 |
| III. *Oxygen* 99, per cent | v/16 | 5 | 58.7 | 67.5 | 3.34 | 9.95 |
| | v/23 | 14 | 37.1 | 45.1 | 4.76 | 10.32 |
| | vi/6 | 9 | 13.8 | 19.7 | 6.95 | 12.02 |

away the cyanide, as well as a bath of Ringer + $O_2$ for a few minutes can accomplish this, and filling the muscle chamber with an atmosphere of 99 per cent oxygen, both tension and heat production increase, the former nearly to, the latter somewhat above, the levels recorded before the anaërobic treatment, and while in only 21 per cent oxygen.

All our muscle-nerve preparations were not sent through the same gamut of changing oxygen pressures, as the one reported in table 1. In the earlier experiments only atmospheric air was used, then in later experiments, this gas-mixture was followed by cyanide and $N_2$-gas; in still later experiments we began first with cyanide and $N_2$-gas then changed from this directly over to 99 per cent oxygen; in another experiment we began with high

oxygen tension and then passed over to zero oxygen tension by the Ringer + CN + $N_2$ route. In the experiment of vi/6 the muscle was kept in high oxygen tension for a longer period than reported in table 1. In this case it is found that the increased heat production gradually diminished until it fell to a level approaching that found for muscle contracting in 21 per cent oxygen. The mean values given in tables 2 and 3 for this experiment do not include these later records in 99 per cent oxygen, since the object in those tables is to show what the *immediate* effect is of the return of an oxygen-hungry muscle to an atmosphere rich in oxygen.

Some other information should still be added. The animals from which the preparations were taken were mostly females, and they being larger than the males supplied the heavier organs; their body lengths varied roughly from 20 to 30 cm. In most of our experiments the durations of stimuli were varied much less than they were in the experiment shown in table 1, and although sometimes, just as in this one, no spontaneous contractions occurred, yet in some, as that of v/25/28, spontaneous contractions were the rule. So long as the strength of current was weak enough we could not observe any certain effect of changing duration of stimulus from 0.25 to 3.0 seconds upon the unit heat production, initial or delayed, nor any difference between the effects of the induced stimulation and those of the intrinsic stimulation that called forth the spontaneous contractions.

The weights of the contracting mass of bladder in the various experiments were taken, somewhat arbitrarily as the weight of the whole organ less ten per cent, the deduction allowing for the remaining part of the neck, and tips of the lobes, in short all those parts of the organ not lying within the contracting points and that could not produce within themselves, or absorb any, of the heat that contributed to the general rise of temperature of the organ during an act of contraction. The weights of the contracting masses of the bladders and their mean temperatures for the various experiments here reported were:

| DATE OF EXPERIMENT | WEIGHT OF MUSCLE | MEAN TEMPERATURE OF MUSCLE-CHAMBER |
|---|---|---|
| | *grams* | °C. |
| iv/11 | 1.89 | 22.0 |
| iv/18 | 2.93 | 21.3 |
| v/2 | 2.13 | 19.5 |
| v/16 | 3.10 | 19.0 |
| v/18 | 2.47 | 21.3 |
| v/23 | 2.29 | 23.0 |
| vi/6 | 0.96 | 23.5 |

The final results of our experiments are gathered together in tables 2 and 3. In these tables are given the extremes of observations, as well as the mean, of both mechanical tension and thermal responses evoked during the contraction of the muscles. As will be seen in table 2 variation among total heats for the various muscles under constant tension of oxygen is

greater than the variations of corresponding tension. This doubtless is due in part to the greater error in the method of measuring the heat production. We are convinced, however, that there are other causes for the variation, that are to be looked for within the muscle tissue itself. In spite of these variations under constant conditions, when the mean values of the total heat production of muscles contracting in the three widely different pressures of oxygen are compared, it is quite clear that the heat liberated is

TABLE 3

*Summary, mean tensions and mean initial, delayed and total heat production in the various experiments*

| TREATMENT OF PREPARATIONS | DATE OF EXPERIMENTS 1928 | NUMBER OF THERMOMYOGRAMS | TOTAL TENSION ($\tau$) | HEAT PRODUCTION IN CALORIES $\times 10^6$ | | |
|---|---|---|---|---|---|---|
| | | | | $H_i/T$ | $H_\tau/T$ | $H_t/T$ |
| | | | *grams* | | | |
| I. *Oxygen*, 21 per cent... | iv/11 | 5 | 32.5 | 1.50 | 2.27 | 3.77 |
| | iv/18 | 14 | 35.0 | 1.97 | 1.93 | 3.90 |
| | v/2 | 2 | 38.2 | 3.25 | 3.89 | 7.14 |
| | v/23 | 7 | 44.5 | 2.63 | 3.54 | 6.17 |
| Mean of 4 experiments | | | | 2.33 | 2.91 | 5.24 |
| Mean of 28 variants | | | | 2.13 | 2.54 | 4.67 |
| II. *Cyanide*, then pure $N_2$-gas..... | v/16 | 8 | 66.9 | 2.53 | 1.33 | 3.86 |
| | v/18 | 5 | 45.9 | 1.69 | 0.41 | 2.10 |
| | v/23 | 9 | 32.6 | 1.58 | 1.02 | 2.60 |
| | vi/6 | 6 | 12.9 | 3.40 | 2.08 | 5.48 |
| Mean of 4 experiments | | | | 2.30 | 1.22 | 3.51 |
| Mean of 28 variants | | | | 2.26 | 1.22 | 3.48 |
| III. *Oxygen*, 99 per cent... | v/16 | 5 | 63.1 | 2.93 | 3.12 | 6.05 |
| | v/23 | 14 | 42.1 | 3.19 | 4.02 | 7.21 |
| | vi/6 | 9 | 15.8 | 3.63 | 5.41 | 9.04 |
| Mean of 3 experiments | | | | 3.25 | 4.18 | 7.43 |
| Mean of 28 variants | | | | 3.28 | 4.31 | 7.59 |

least of all for the muscle contracting in complete absence of oxygen, and most of all for those oxygen-hungry muscles just returned to an atmosphere of 99 per cent oxygen.

Comparing first the mean result for total heat produced by this smooth muscle in atmospheric air with what has been observed for other muscles of the same species of animal we find that the figure, 52 microcalories, for the H/T ratio, is nearly one-third less than the mean found for the cardia

sphincter of the stomach (1927) and from one-third to nearly one-half less than was found for the ventricle and auricle. (For summary of these latter see Snyder, 1928, p. 81, ff, and Gemmill, 1927.) Only when the bladder muscle is recovering from oxygen-want in a high tension of oxygen does its total heat production become as high as has been found for the other muscles.

From the mean results in table 3 we may arrive at the additional absolute amounts of heats liberated, due to the presence of oxygen, by subtracting the mean values found during anoxybiosis from those found during oxybiosis. The latter condition when not preceded immediately by a period of anoxybiosis, as we have seen, yields less heat than when it is preceded by oxygen-want. Let us assume the condition in atmospheric air as shown in the first section of table 3 to be more nearly the normal condition for oxybiosis. The mean values of this period subtracted from those in the next section of the table, containing the results of muscle treated in cyanide and left in pure $N_2$-gas, will then give what we may consider the more nearly normal amounts of heat developed in the presence of oxygen during a cycle of muscle action. Thus, taking the mean of the experiments, in calories $\times 10^5$:

| | INITIAL | DELAYED | TOTAL |
|---|---|---|---|
| During oxybiosis, 21 per cent oxygen | 2.33 | 2.91 | 5.24 |
| During anoxybiosis (heat of anoxidative origin) | 2.30 | 1.22 | 3.51 |
| Additional heat in the presence of oxygen | 0.03 | 1.69 | 1.73 |

From this it appears that the "oxidative" initial heat is practically a negligible part of the total initial heat, as was found also for the ventricle, being only 1.3 per cent in the former, about 5 per cent in the latter, both quantities well within the experimental error. The anoxidative delayed heat on the other hand is about 42 per cent of the total delayed heat, whereas for ventricle muscle it was found to be nearly 15 per cent. The ratio of delayed anoxidative to initial anoxidative is nearly as 1:1.9 while this ratio for ventricle was found to be 1:3.0. In other words, the smooth muscle of urinary bladder produces, independently of the presence of free oxygen, a good deal more heat during the phase directly following contraction than does the ventricular muscle, whereas during the contraction period practically no heat depending upon oxygen is produced in any of the muscles.

Oxybiosis in an atmosphere of nearly pure oxygen following upon a prolonged period of absolute oxygen-want, on the other hand, shows a marked increase in both initial and delayed heats of contracting smooth muscle, as

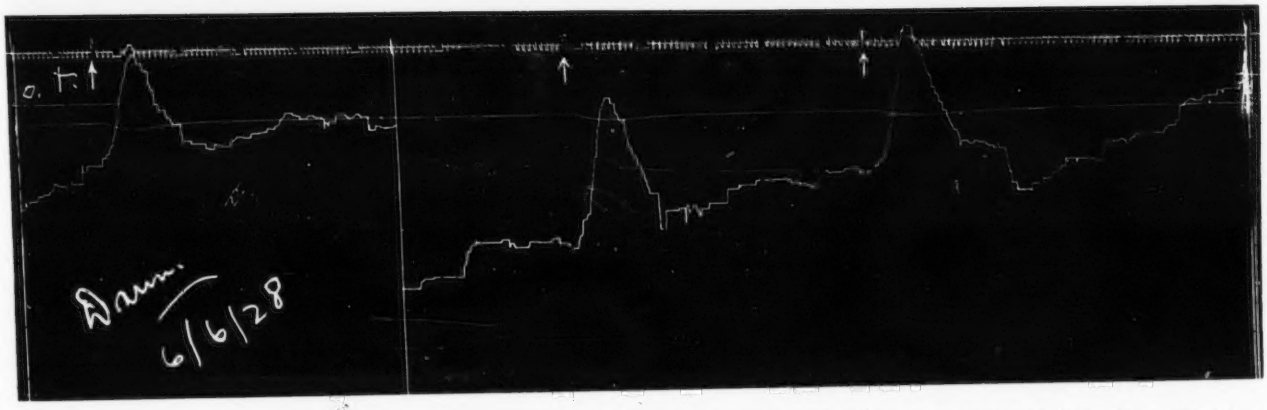


Fig. 3. From the experiment of ~~3~~ 6/28. Thermomyograms of the terrapin urinary bladder nerve preparation in an atmosphere free from oxygen. The preparation had been subjected to a bath of oxygen-free Ringer + m/3000 CN ions for 7 minutes, the Ringer then displaced with pure $N_2$-gas and the preparation allowed to rest for 42 minutes. Temperature 23.5°C. Smaller time-marks are for 3 second intervals, the larger roughly for 30 second intervals. At the arrow-points the nerves were stimulated faradically, (secondary coil at 12 cm., 1 dry cell in primary) for 3 seconds to evoke the first and second contractions, 1.5 seconds to evoke the third. The short line below *o.t.* at left indicates zero tension of the tension lever (M), downstroke of which indicates rise of tension. Note the large rise of temperature (upstroke of $\theta$) during the muscle contraction and, compared with that in figure 2, the relatively small rise of temperature during the relaxation of the muscle. The galvanometer spot ($\theta$) shows a slow drift to the right of scale,—upward on record

was found also for ventricle. The mean values of the experiments from table 3, again in calories $\times 10^5$, are:

| | INITIAL | DELAYED | TOTAL |
|---|---|---|---|
| In oxybiosis, 99 per cent oxygen, following anoxybiosis | 3.25 | 4.18 | 7.43 |
| In anoxybiosis (anoxidative heat) | 2.30 | 1.22 | 3.51 |
| Additional heat in the presence of 99 per cent oxygen | 0.95 | 2.96 | 3.92 |

An oxygen-hungry urinary bladder muscle when given an atmosphere of nearly pure oxygen, but under otherwise similar conditions, thus produces a total heat nearly 42 per cent greater than muscles in atmospheric air and that have not been subjected to a prolonged period of oxygen-want; the amount now approaches the figure that was found for ventricle in 21 per cent oxygen. The initial "pure oxidative" heat is nearly 29 per cent of the total initial, and the pure anoxidative delayed heat curiously is also about 29 per cent of the total delayed; these figures being 1.3 and 42 per cent respectively for muscle not recovering from prolonged oxygen-want.

These mean results are shown graphically in figure 4, where along a time axis (about six minutes in duration) the rise and fall in muscle tension are plotted by a conventional contraction curve. From the time axis and along the ordinates are erected blocks of heat the heights of which represent the relative amounts liberated during the two major phases of tension change, and under the three conditions studied, namely, *a*, muscle surviving in complete anoxybiosis; *b*, the oxygen-hungry muscle upon being returned to the oxybiotic condition (or "recovering") in 99 per cent oxygen; *c*, the unfatigued, fairly well oxygenated muscle surviving in 21 per cent oxygen. In the diagram, *a* is represented by the double-hatched portion of the block, *b* by the extent to which the unshaded portions reach up above all the rest, *c* by the extent the single-hatched portions of the blocks pass beyond the double-hatched parts. The actual areas of the blocks have no significance.

As to the later thermal behavior of these muscles after experiencing a period of high oxygen tension, the following two examples are instructive. The experiment of vi/6/28 was one in which the preparation first was put in the muscle chamber at 10:30 a.m. At 3:00 p.m. it had been in oxygen-want, following a cyanide treatment, for 2.25 hours whereupon the nitrogen gas was displaced with Ringer-$O_2$ and this in turn with 99 per cent oxygen. The heat production that could be measured from contractions up to the end of 1.5 hours of this oxybiosis, as shown in tables 2 and 3 for this experiment, exhibited the usual high values for muscles brought from zero to high oxygen pressures. At 4:30 p.m. the 99 per cent oxygen was with-

drawn by way of the Ringer-$O_2$ route and the muscle-chamber filled with atmospheric air. Of the 13 that could be recorded by 6:00 p.m., the latter 9 thermomyograms show H/T ratios of an order approaching the previous values for muscles in 21 per cent oxygen. Summarizing the mean thermal behavior of this muscle in the sequences of its experiences with the changes in oxygen-tensions we have these heat-productions, in cal. $\times 10^5$:

In zero oxygen tension, initial 3.40, delayed 2.08
In 99 per cent oxygen, initial 3.63, delayed 5.41
In 21 per cent oxygen, initial 2.75, delayed 3.45

From this it appears that the increased heat production in oxybiosis for at least the first 90 minutes following complete anoxybiosis is a function

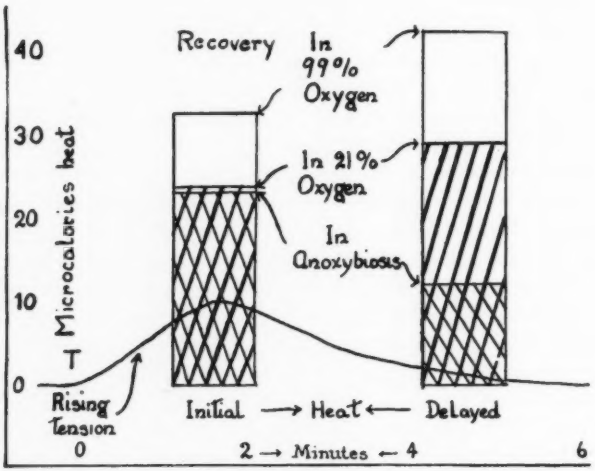


Fig. 4. Initial and delayed heat during the single contraction wave in smooth muscle. See text for description.

of the oxygen tension. But we could hardly expect this increased heat production to continue indefinitely even in 99 per cent oxygen, a surmise that is fully confirmed by the experiment of v/25/28. This muscle after having been treated with cyanide and kept in pure nitrogen for 2.5 hours, was then exposed to 99 per cent oxygen for 2.3 hours. The muscle exhibited slow spontaneous contractions at the end of this time (as it had done earlier) and nine thermomyograms were obtained during the next half-hour, the initial and recovery waves of which were distinct and measurable. One and a half hours later another lot of records was taken, 5 contractions, all spontaneous, being obtained during another half hour.

The tensions and heat ratios *a*, for the first five thermomyograms of the first period, and *b*, the last five thermomyograms of the second period in 99 per cent oxygen, stated again in calories $\times 10^5$, are:

| | TENSION | INITIAL | DELAYED | TOTAL |
|---|---|---|---|---|
| | *grams* | | | |
| After 2.3 hours | 56.5 | 2.66 | 4.52 | 7.16 |
| After 4.0 hours | 57.7 | 1.74 | 2.99 | 4.73 |

In this case it is clear that the large heat ratios observed during the early hours of recovery from oxygen-want begin to decrease, even when the muscle is kept in 99 per cent oxygen, until the low levels observed for fresh muscles in atmospheric air are reached. We did not continue our experiments as a general rule beyond 12 hours on account of the liability of damaging our thermopiles if kept wet for long periods of time.

Our analyses thus far have had to do with clonic contractions, but since tonus is exhibited so markedly by the smooth variety of muscle, the question as to what extent that factor enters into our results naturally arises. All our graphic records contain the data for determining the initial tension of muscle, that is, tension maintained just before and at the end of the clonic contraction curves. From these data the tonic condition of the muscle can be stated in absolute values. Many of our tabular analyses contain these values and we have compared them for the different myograms both when conditions were kept constant and when the oxygen tension was varied. So far, we have met with no evidence indicating that tonus, that is, initial tension, in any way affects the magnitude of the waves of heat-production during the intervening clonic contractions. However, our records do confirm the observation of Hoskins and Hunter (1924), among others, that smooth muscle deprived of oxygen exhibits a lesser degree of tonus than when supplied with an abundance of oxygen. Thus in two experiments the initial tensions, stated as the mean of the two extremes of muscle lever excursion:

Experiment of v/23, when in CN + $N_2$, 6.7; when in 99 per cent oxygen, 9.2
Experiment of v/25, when in CN + $N_2$, 9.9; when in 99 per cent oxygen, 13.7

The increase of tonus for the muscle in oxybiosis in these two cases is [redacted] 37.3 and 38.2 per cent respectively.

DISCUSSION. With the knowledge we have now gained concerning the thermal behavior of smooth muscle, let us proceed to a more general comparison of the results with what is known of the other two major contractile tissues, and a brief discussion as to their relation to the known processes involved in the act of contraction. With this end in view we have prepared

a table of the mean relative values of the known heat productions, in the principal phases of muscle response to stimulation, in which the initial anoxidative heat of bladder muscle is made equal to unity. The quantities here are for unfatigued muscle:

| KIND OF MUSCLE* | INITIAL | | DELAYED | | TOTAL |
|---|---|---|---|---|---|
| | Anoxidative | Oxidative | Anoxidative | Oxidative | |
| Bladder | 1.00 | 0.05 | 0.54 | 0.77 | 2.36 |
| Ventricle | 1.04 | 0.04 | 0.35 | 2.00 | 3.43 |
| Sartorius | 1.06 | † | 0.32 | 1.23 | 2.65 |

* For the data on bladder see the table on p. 413 of this article for the anoxybiotic values; for the values in oxybiosis in 21 per cent $O_2$ we have included the data of exp. iv/6 mentioned on page 415, the mean values now taken are for 5 instead of 4 experiments. The data for ventricle are from Snyder, 1928, p. 86. The data for sartorius are taken from the papers of Hartree and Hill, the absolute and relative values being put on the common $H/T$ basis as estimated by Snyder (1926, p. 176) from the original data.

† No statement is made of initial oxidative heat for sartorius other than there is none so long as advanced fatigue is avoided (Hill and Hartree, 1920, p. 106).

From these relative values the following statements may be made: 1. Just as in skeletal and cardiac, so in smooth practically the whole of the initial heat is quite independent of the presence of free oxygen. Now since the absolute quantity for smooth muscle is so nearly equal to that found for skeletal it becomes probable that the immediate processes, whatever they may be, producing tension-rise in smooth muscle are the same in kind, if not in degree, as those occurring in skeletal muscle. This interpretation is supported by the newer knowledge of the chemistry of smooth muscle. For example C. L. Evans (1925) finds in isolated guinea-pig uterus, during anaërobic survival, that the lactic acid content increases and the carbohydrate (including glycogen) content decreases. These initial processes in smooth muscle, then, most probably consist of anoxidative mobilization and fermentative breakdown of carbohydrates, quickly followed by ionization of acids and protein, substitutions and deionizations, as finally established by Embden and by Meyerhof. (For a recent review of the latter's work, in part, see Snyder, Science, 1927, lxvi, 112.)

2. The small differences that appear in the quantity of total initial heat production by the unfatigued muscle in oxybiosis seems to be in part due to a small quantity of heat of an oxidative origin. Time being an important factor for heat of this kind to accumulate, it is not surprising that the more slowly contracting muscles, terrapin bladder and ventricle, show a positive amount of initial oxidative heat. That none appears in skeletal muscle must be due to the comparatively brief period of the phase of contraction in

that muscle, the durations of the contraction phase of the three classes of muscle, as is well known, being roughly 30, 0.3 and 0.03 seconds respectively at 20°C.

3. But aside from this difference in initial heat of an oxidative source, there is also a small increase in the anoxidative quantity as one passes from smooth to skeletal muscle. In other words, so far as the anoxidative initial heat is concerned, the slower its contractions the more efficient a muscle becomes. To account for this one recalls the hypothesis proposed and developed by Gasser and Hill (1924) which stresses the rôle played by the viscosity of muscle fluids in the efficiency of the contractile act of skeletal muscle. A migration of fluid or particles within the fluid being assumed, the resistance due to viscosity increases inversely with the duration of contraction. Therefore, to develop equal quantities of tensions, the more slowly contracting muscle requires a smaller quantity of energy. It may well be that smooth muscle, in its more leisurely development of energy, sets up less viscous resistance and, therefore, has a greater remainder of energy to convert into tension.

4. Turning our attention now to the delayed heat productions of these three muscles we note again a close agreement of the anoxidative delayed heat between skeletal and cardiac (as pointed out by Snyder, 1928). A similar but even larger anoxidative delayed heat appears in the smooth muscle. Why this anoxidative heat is greater in smooth muscle than in cross-striped we can not now say; indeed the source of the smaller amount in cross-striped muscle is unknown. The fact that it appears in considerable quantity in smooth as in cross-striped points rather to a difference in degree rather than in kind of the underlying processes, whatever they may be.

5. In the case of the oxidative delayed heat we find the greatest divergence. When we recall that it is this portion of the total heat that is held to be responsible for the "recovery" processes, and that this in turn depends upon the amount of fatigue products accumulated in the muscle, or more precisely, the amount of these products still remaining in the tissues from previous contractions and from previous oxygen-want, and when we recall that this period covers by far the longer period of time, it is not surprising at all that this portion of heat of the muscle's whole action should show the most variation. The comparison of the heat production at various stages of a muscle recovering from anoxybiosis in pure oxygen, as shown above, in the experiment of v/25, shows that the factor of time relations is important. We happened to catch that muscle just at the turn and could demonstrate the change. It may be that, although one tried to work with muscles unfatigued, there nevertheless remained among them various quantities of fatigue products. This is especially true probably for the

ventricles whose walls had been cut from apex to base in preparing them for the thermopile; among them trauma always was at hand.

A comparison of the relative mean results of fatigued bladder and ventricle recovering in 99 per cent oxygen with those of unfatigued sartorius (if not in pure, at least in excess of, oxygen) will be instructive on this point. In doing this the initial anoxidative heat of unfatigued bladder again is put equal to unity.

*Oxidative heats**

| MUSCLE | INITIAL | DELAYED | TOTAL |
|---|---|---|---|
| Bladder, recovering | 0.41 | 1.28 | 1.69 |
| Ventricle, recovering | 1.04 | 3.50 | 4.54 |
| Sartorius, unfatigued | | 1.23 | 1.25 |

* For the absolute values on ventricle see Snyder (1928, pp. 83 and 84); those for bladder, above on page 415; those for sartorius, the same as quoted above.

Here we see again how promptly the oxygen-hungry muscle utilized not only the period after but also the period during contraction to rid itself of accumulated fatigue products of former contractions.

What we wish to emphasize here is that it is only during recovery from prolonged anoxybiosis that the smooth muscle develops as much oxidative heat during the period immediately following contraction as does the unfatigued sartorius. On the other hand under the same conditions ventricle produces nearly three times as much as bladder muscle. Although no data are at hand the inference is unavoidable that fatigued skeletal muscle also increases its delayed oxidative heat just as promptly as does cardiac and possibly even more promptly.

It must then be concluded that the oxygen-hungry smooth muscle while increasing its quantities of oxidative heats both initial and delayed still lags behind the cross-striped muscle. That this lag, however, in both the initial and delayed stages is very nearly the same (being nearly 26 per cent of the cardiac amount in both phases) is a fact probably significant of a fundamental difference in the two muscles in, say, the kinds or quantities of oxidative catalysts necessary to hasten the oxidative processes. But this apparently would be a factor of mere adaptation. A muscle so sluggish and so rarely called upon to make a single contraction as is that of a turtle's urinary bladder would have no need of the same kinds or the same quantity of oxidative catalysts as would the heart muscle of the same animal, beating 20 times a minute, or the hind-leg muscles of a lively frog. It may well be therefore that, so far as our experiments are concerned, in the period we gave for the measurement of the whole of the delayed heat we either allowed too short a time for that phase to be completely registered,

or the processes themselves trailed off into waves of such slow rate that our apparatus failed to register them any longer.

Such considerations as these, therefore, must be thoroughly tested out before one concludes upon the evidence of our observed delayed heat, that the recovery processes of smooth muscle must be different from those of cross-striped muscle.

## SUMMARY AND CONCLUSION

1. A method is described for the isolation in Malaclemmys palustris of its urinary bladder with the peripheral ends of two pairs of motor nerves intact. An arrangement is further described whereby the smooth-muscle-nerve preparation thus obtained may be set up for recording simultaneously the mechanical and thermal responses evoked by stimulation of the nerves.

2. Experiments are described where the muscles were made to contract in atmospheric air, in pure nitrogen following treatment with cyanide in oxygen-free Ringer, and in 99 per cent oxygen. Records of tension and thermal responses in both the major phases of contraction, during both oxybiosis and complete anoxybiosis were thus obtained, analysis of which led to the following conclusions:

a. The mean absolute initial heat produced by the smooth muscle of this organ is nearly all anaërobic in origin and, per gram muscle and per gram tension, is of the same magnitude as has been found for skeletal (A. V. Hill and associates) and for cardiac (C. D. Snyder) from which it follows that the processes leading to contraction in smooth muscle, when considered in conjunction with the newer knowledge of the chemistry of this muscle, are probably the same as they are in cross-striped muscles.

b. As has been observed in skeletal and cardiac muscles there is liberated also in smooth muscle a quantity of delayed anoxidative heat, the mean value of which in four experiments however is considerably greater than that found in the cross-striped varieties.

c. The delayed oxidative heat production of the bladder muscle, only when recovering, in oxygen of high tension, from a previous prolonged anoxybiosis, has a mean value equal to that observed for unfatigued sartorius. When unfatigued, or recovery is apparently near completion, the delayed oxidative heat of the smooth is of a smaller magnitude, as a rule, than that found for the cross-striped muscle.

d. During the early period of recovery from prolonged anoxybiosis, an oxidative initial heat production is observed for the smooth, as well as was observed for cardiac muscle.

3. Finally, the significance of these various oxidative and anoxidative heats in their relation to the underlying processes of muscle contraction is briefly discussed.

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# STIMULATION OF NERVE AT THE ANODE WITH SHORT INDUCTION SHOCKS

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Received for publication July 2, 1928

In the literature Garten (1909), Fick (1871) Thörner (1922) and others have presented indirect evidence to indicate that in stimulation of a nerve with induction shocks there is a response at the cathode on the rise of the potential, a response at the anode on the fall. The physiological effect from an induction shock would thus be similar to that from a galvanic current. This evidence was obtained by noting the presence of a second wave in the falling phase of a nerve action potential recorded by a string galvanometer after conduction or by an analysis of the changes in the form of the muscular response with increasing strengths of stimulation. It is the purpose of this paper to investigate the conditions at the site of stimulation as a check on these observations. This has been made possible by the use of the cathode ray oscillograph as a recording mechanism which permits the precise recording of very rapid potential variations.

While carrying out investigations on the effect of anoxemia in nerves it was noted that in those quickly excised and mounted in air at the beginning of an experiment the action potential wave was of much longer duration than it was after an interval of time had elapsed, or almost immediately after surrounding the nerve with hydrogen, nitrogen or carbon dioxide. This shortening of the duration of the action potential wave was seen to be in part at least due to the dropping out of one or more waves which occurred in the falling phase of the main wave or usual cathodal response. These multiple waves were obviously not those resulting from the separation out of the action potential waves of different fiber groups after conduction demonstrated by Erlanger, Gasser and Bishop (1924) because they were obtained practically at the point of stimulation.

To determine the nature of these multiple waves at the stimulus two series of experiments were ultimately performed. In the first series short break induction shocks were used to stimulate, in the second a galvanic current of short duration. The induction shocks, 0.06 sigma in effective

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duration, were applied through platinum electrodes from a coreless Porter coil. The source of primary current was a two volt wet cell distributed through a rotating commutator. The strength of applied current was variable by means of a slide wire of 6 ohms resistance in series. To lessen the size of the "escape" the primary current was shunted by 100 ohms. The galvanic current source was a two volt wet cell distributed through a rotating commutator, the potential variable by means of a slide wire potentiometer. The nerve preparation was balanced for resistance in a Wheatstone bridge in the manner described by Bishop (1927).

As leads to the recording apparatus platinum electrodes were used in the majority of the induction shock experiments after demonstrating that the results were indistinguishable from those obtained with non-polarizable

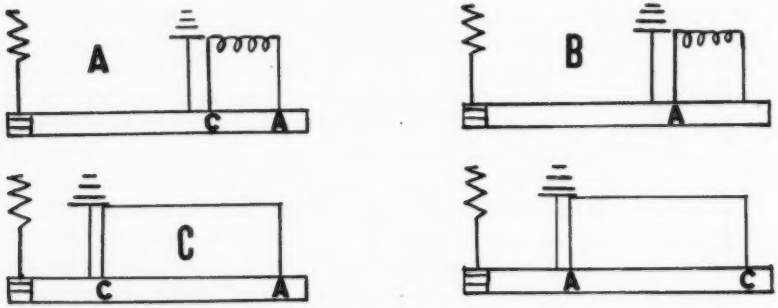


Fig. 1. Scheme of experiments following. A—nerve preparation mounted for induction shock experiment. $C$ = cathode, $A$ = anode; grid lead on killed end of nerve; descending current. B—same with anode proximal to ground lead; ascending current. $C$ = nerve preparation mounted for galvanic stimulation; cathode proximal. $D$ = same with anode proximal.

electrodes. The amplifier employed had such a high input impedance that the current density through the electrodes was insufficient to polarize them appreciably. Non-polarizable electrodes were used in stimulating by galvanic currents, though this precaution also was probably unnecessary on account of the short duration of the currents.

*Induction shock experiments.* As materials sciatic nerves of the green frog and bullfrog and bullfrog nerve roots were employed. These were mounted as indicated in figure 1, A and B. The distance between the stimulating electrodes was variable. The results obtained with the conditions as in figure 1 A will first be considered, where the stimulating cathode is proximal and close to the leads. Just above threshold a single action potential wave as shown in figure 2 A is seen. On increasing the strength of stimulus moderately a second wave appears in the falling phase

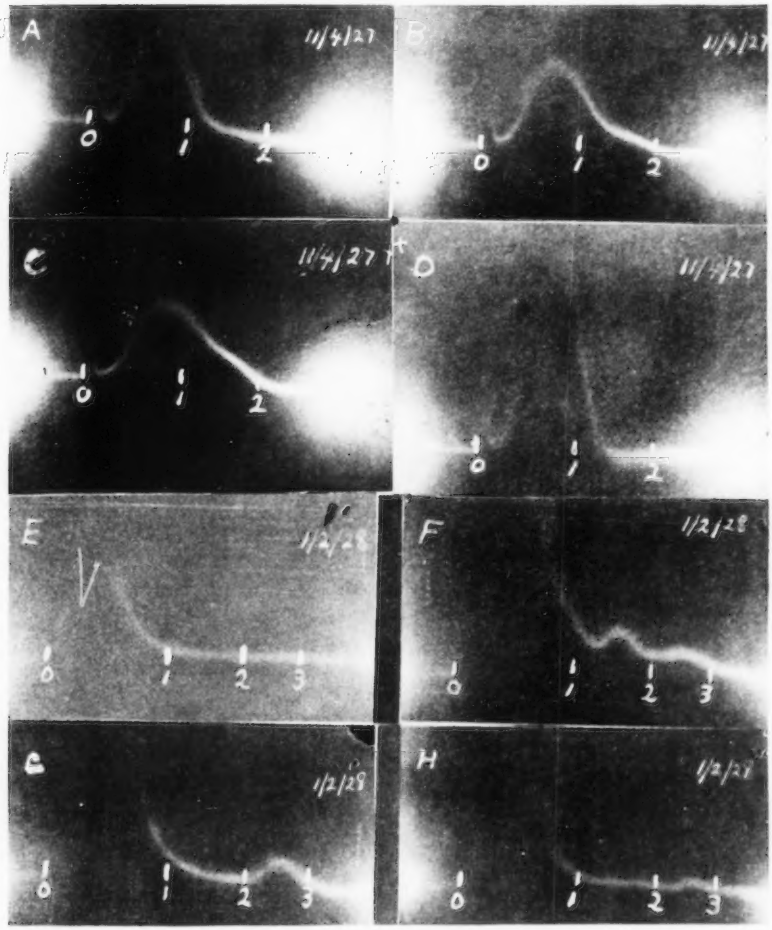


Fig. 2. Films A, B, C, D. Action potentials green frog sciatic with increasing strength of induction shocks (0.06 $\sigma$) in the order given, cathode proximal in all; time in sigma. A—single cathodal wave. B—cathodal wave followed by anodal wave. C—anodal wave increased in amplitude and slightly earlier. D—cathodal wave only when stimulus maximal, anodal wave blocked.

Films E, F, G, H. Action potentials green frog sciatic with increasing strength of induction shocks in order given; cathode proximal. Proportional threshold strengths 132, 152, 156, 192. E, single cathodal wave. F, cathodal wave followed by 4 anodal waves, the last three being repetitive. G, slowing of repetitive anodal wave due to depression under cathode. H, depression of repetitive anodal responses by catelectrotonus.

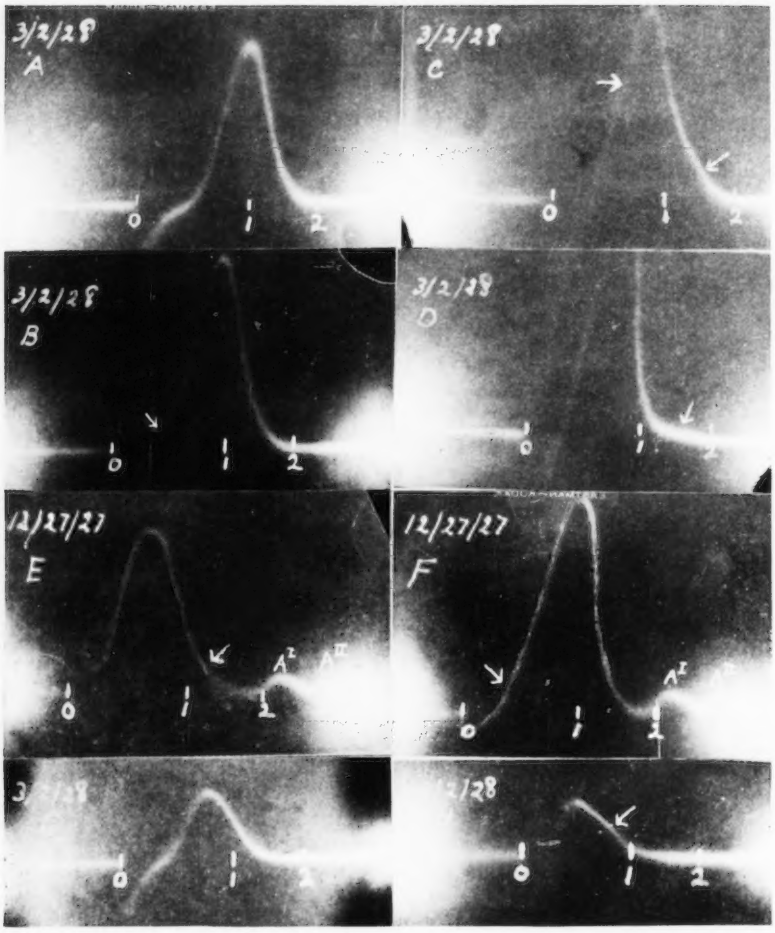


Fig. 3. Films A, B, C and D. Action potentials from green frog sciatic with increasing strengh of induction shocks (0.06 $\sigma$), anode proximal in all; 9 mm. between electrodes; 3 mm. conduction; time in sigma. A—single cathodal wave, primary current 164. B—two waves, 1st low anodal indicated by arrow, 2nd cathodal, stimulus 188. C—two waves, 1st anodal, 2nd cathodal, waves indicated by arrow, stimulus 252. D—two waves, 1st anodal, 2nd indicated by arrow low cathodal, stimulus 520. Cathode wave almost blocked.

Films E and F (retouched). Action potentials from green frog sciatic; stimulus same for two potentials. E—cathode proximal. F—anode proximal. Note cathodal wave is earlier in E than in F whereas anodal waves A I and A II are both later. Arrow indicates anodal wave succeeding or preceding and summed with the cathode wave.

Films G and H. Action potentials from green frog sciatic 9 mm. between electrodes, 3 mm. conduction. 34,000 ohms shunt. G—anodal wave only (cathodal blocked); H—cathodal wave originating at ground electrode followed by anodal wave (indicated by arrow) when stimulus is increased sufficiently to spread to ground electrode.

of the first (fig. 2 B) or sometimes later than the falling phase of the first, depending on the conduction rate of the nerve and the distance between the stimulating electrodes. The amplitude of this second wave increases with increasing strength of stimulus to its maximum. It may in turn be followed by a second or third wave similar to the original first wave but lower in amplitude (fig. 2 F). Each of these waves has a definite threshold. Four waves following a first wave is the highest number so far observed. When the stimulus becomes maximal or supramaximal for a cathodal response the first secondary wave may be blotted out (fig. 2 D) and later waves persist with their conduction rate delayed (fig. 3 G) and amplitude lowered (fig. 2 H).

When the direction of the stimulating current is reversed (fig. 1 B) it is again possible to obtain multiple waves on increasing the strength of the stimulating current. Figure 3 A shows the usual single first response which on increasing the strength of stimulus is first preceded by another wave (fig. 3 B, the summed wave, starts earlier but lasts as long as A) and on further increase the original wave decreases as the other increases (fig. 3 C and D). At this stage new repetitive waves may appear later in time (fig. 3 E and F). Finally when the current is maximal or supramaximal the original wave entirely disappears leaving only one or more of the new waves.

It is essential to note that multiple waves can be elicited with short induction shocks in nearly all freshly excised normal nerves. The more irritable nerves (low threshold for cathodal response) show a series of waves while those less irritable show only a single second response. Evidence has shown them to appear less frequently in summer than in winter frogs. On standing in moist air, on preservation in Ringer's solution or in an atmosphere deprived of oxygen the ability to develop second responses is lost. When present in good preparations they persist from ten to at least ninety minutes. The nerves employed were not cooled, all our experiments being carried out at room temperature (20 to 25°C.).

Analysis of the conditions underlying these secondary responses necessitated a consideration of factors known to produce waves or artefacts in the usual action potential record. Bishop, Erlanger and Gasser (1926) demonstrated that such artefacts could arise from nerve branches when found between the stimulating electrode and the ground lead to the recording apparatus, due to double stimulation, or between the recording leads, due to effective double leads. This as a causative factor was ruled out in our experiments by utilizing portions of the nerve without branches and especially by recording from bullfrog nerve roots. Second waves were obtained from all portions of the nerve length. They were especially easy to elicit when the anode was near the freshly cut end of the nerve

preparation. The cathodal response on the other hand in this region became quickly depressed on repeated stimulation.

This observation is consistent with the idea that the complex potential wave might be due to stimulation at the anode as well as the cathode. Separation of the stimulating electrodes should therefore tend to separate the waves. This was found to be the case. By observing the differences in conduction time of the waves due to separation of the electrodes it was possible to demonstrate that the repetitive responses invariably arose at the same electrode, the anode. The cathodal response was always found to be single. By simply altering the direction of the stimulating current the resultant differences in conducting distance permit the demonstration of these facts. In figure 3 E the stimulating cathode is proximal, in 3 F it is distal to the recording leads. In F the first or cathodal response is later while the two accessory waves are both earlier than in the first record (E) by an interval corresponding to the time necessary for an action potential to travel from one stimulating electrode to the other.

Direct observation of the changes in form and amplitude of the composite potential wave on alteration of the strength of the stimulating current served to still further check our results. On increasing the strength of the stimulating current in those cases where two waves were present after they had reached a certain amplitude a further increase in amplitude of the earlier resulted in a corresponding decrease in amplitude of the later and vice versa on decreasing the strength of stimulating current. Since it has long been known that two processes travelling in the same fiber in opposite directions block each other when they meet, it followed that the inference between the first and second waves must be due to stimulation of the same fibers at two different points. The waves were too little separated in time in those experiments where the stimulating electrodes were close together to be explained as a repetitive response at one electrode (fig. 3 A-D). The only two points possible were the anode and cathode. These points must therefore cause stimulation in different sets of fibers until the strength of the stimulating current is such as to stimulate all fibers under the one pole nearest the recording electrodes.

Attention must be called to a possible happening with strong stimuli when leading to the recording apparatus from a point close to one of the poles of the stimulating electrode. Gradual spread of these strong stimuli occurs with a resultant decrease in conduction time until finally rather suddenly the current surges to the ground recording electrode and stimulates there. When the cathode is proximal the ground electrode may stimulate as anode, when the anode is proximal the ground electrode may stimulate as cathode giving in each case a double wave. The phenomenon is apparently due to current surging from the capacity of the coil winding to the ground and back. The time relationship of waves thus produced

is, however, reversed to that in which they occur when stimulation occurs at the cathode and anode of the applied stimulating electrodes. Such an occurrence is illustrated in figure 3 G and H. It was at first considered that it would be possible to differentially alter irritability at the cathode or anode by heating between the electrodes, the junction between the killed region and live tissue becoming the effective electrode. The experiments, however, demonstrated that no adequate differentiation resulted.

TABLE 1

*Order of stimulation in nerve with induction shocks*

| INCREASING STRENGTH OF STIMULI | DESCENDING CURRENT | | ASCENDING CURRENT | |
|---|---|---|---|---|
| | Cathode | Anode | Cathode | Anode |
| I | + | − | + | − |
| II | + | + | + | + |
| III | + | + (+) (+) | + | + (+) (+) |
| IV | + | − (+) (+) | − | + (+) (+) |

Descending current = cathode proximal to recording electrodes.
Ascending current = anode proximal to recording electrodes.
(+) Repetitive anodal response.

TABLE 2

*Comparison of strengths of threshold stimuli for cathodal and first anodal responses and strength of stimulus necessary to eliminate cathodal or first anodal response*

| DESCENDING CURRENT = CATHODE PROXIMAL | | | ASCENDING CURRENT = ANODE PROXIMAL | | |
|---|---|---|---|---|---|
| Cathodal response | First anodal response | Stimulus to eliminate first anodal response | Cathodal response | First anodal response | Stimulus to eliminate cathodal response |
| 100 | 112 | 200 | 100 | 132 | 637 |
| 100 | 194 | 326 | 100 | 120 | 667 |
| 100 | 112 | 360 | 100 | 170 | 620 |

Threshold for cathodal response arbitrarily = 100 in terms of current of primary circuit.

On the basis of stimulation occurring at the cathode (single always) on the rise, at the anode (single or repetitive) on the fall of the induction shock potential and keeping in mind the fact that one impulse blocks another in the same fiber, it is not difficult to analyze our experimental findings. With the cathode proximal to the leads to the recording apparatus the second wave arises at the anode. On increasing the strength of stimulus the two processes increase in amplitude and move earlier (due to spread of stimulating current). On further increase of stimulus in irritable nerves the response at the anode becomes repetitive (Ritter's tetanus). When the stimulus is maximal or supramaximal all fibers are

stimulated first at the cathode so that the first response from fibers stimulated at the anode is blocked. Repetitive responses at the anode are, however, not blocked after the cathodal response has passed the anode. Due to catelectrotonus with strong currents these may be depressed in amplitude and somewhat delayed in conduction rate. When the current is reversed and the anode is proximal the anodal response first to appear precedes the cathodal response because the time taken for the cathodal response to reach the ground electrode is longer than that for the anodal response. The closer together the stimulating electrodes are placed the

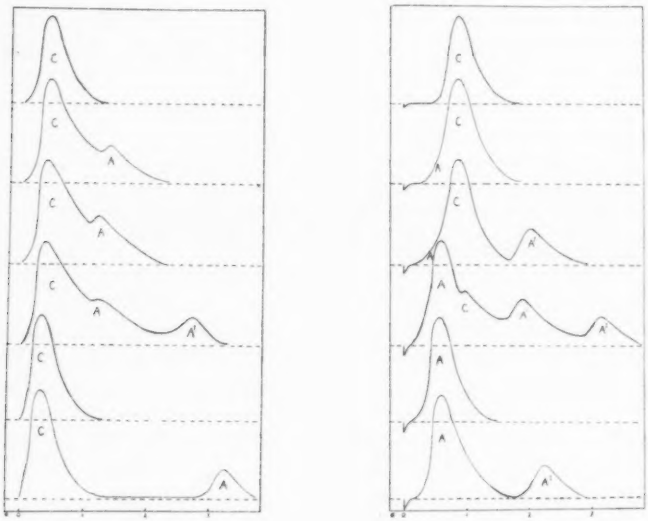

Fig. 4. Diagrams (on linear coördinates) to illustrate the order of stimulation in nerve with short induction shocks and increasing strengths of stimulating current. On the left side cathode proximal, on the right anode proximal; strength of stimulating current increasing from above down. C—cathodal response, A—anodal response, A′—repetitive anodal response.

more will two responses, one from the anode and one from the cathode, tend to merge no matter what the direction of the stimulating current. It is for this reason that some really double action potential waves are apparently simple. Their double nature can be detected by the broadening and delay of their crests. As the stimulus is increased, the response at the anode may become repetitive giving one or more waves later than the cathodal response as well as the one earlier. When the stimulating current becomes still stronger and more and more fibers are stimulated at

the anode the cathodal response is depressed and finally entirely eliminated due to fiber blocking. The only remaining response recorded, single or repetitive, is now from the anode, which, due to anelectrotonus of strong currents, may be somewhat depressed.

The order of stimulation in nerve with induction shocks as found in our experiments is summarized in table 1. Table 2 compares the strength of stimulus necessary for a first anodal response with that required for a cathodal response and indicates the current strengh necessary for cathodal and anodal blocking. Figure 5 shows graphically on rectangular, linear coördinates the order outlined in table 1.

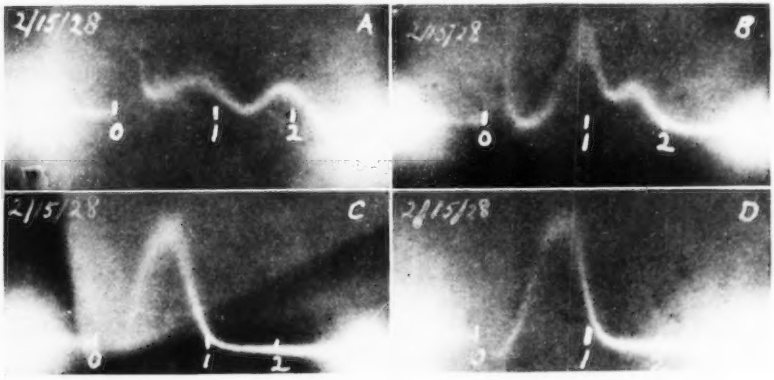


Fig. 5. A—action potential bullfrog sciatic; 3.4 cm. between stimulating electrodes; galvanic stimulus (0.1$\sigma$ duration) just above threshold for cathodal and anodal responses; cathode proximal. B—same with anode proximal. C—cathodal response bullfrog sciatic; 7.5 cm. between stimulating electrodes; galvanic stimulus maximal (0.1$\sigma$ duration) cathode proximal; 0.5 cm. conduction; anodal wave blocked by maximal cathodal wave conducted toward it. D—anodal response from preparation C on reversal of stimulating current, i.e., anode proximal; cathodal wave blocked.

*Galvanic current experiments.* A nerve will respond with single or repetitive responses, at the cathode on the make of an adequate galvanic current, at the anode on the break of such a current. It was considered pertinent to our investigation to determine how brief a galvanic current could be and still give findings comparable to those usually obtained. The nerves were mounted as indicated in figure 1 C and D with rather wide (3.5 to 7 cm.) separation of the stimulating electrodes, to give adequate separation of anodal and cathodal responses.

The results will not be given in detail as they are a repetition of those described for induction shocks. It was found possible to obtain repetitive

anodal responses with currents as brief as 0.1 sigma. Figure 5 A shows an anodal wave obtained with the cathode proximal to the recording leads, figure 5 B with the anode proximal. Figure 5 C and D shows respectively a single cathodal response and a single anodal response obtained through maximal stimuli with the direction of the stimulating current first descending and then ascending. The two are identical except for the fact that the second is later by approximately the duration of the stimulus. The demonstration that with submaximal stimuli the cathodal and anodal responses arise in different fiber groups was again convincing. Consequently it can be said that the short galvanic stimulus and the short induction shock are essentially similar in that both have a bipolar effect on the nerve.

No attempt will be made to theorize as to the mechanism of stimulation at the anode for single or repetitive responses. The old interpretation of Matteucci (1867) in which he considers stimulation at the anode a result of the make of the polarizing current seems as adequate as any. But it is difficult to understand how polarization at the anode can be of sufficient duration to act as a constant current stimulating repetitively. With induction shocks as with galvanic currents the threshold for stimulation at the anode is always higher than for stimulation at the cathode. The more irritable the nerve the less the difference between the two strengths of threshold current. Polarization of the nerve sheath is not a deciding factor in anodal stimulation as shown by our results in bullfrog nerve roots, but the myelin sheath may be the structure involved.

Brief reference may be made to the work of Garten (1909) and Forbes and Gregg (1916). Both of these investigators obtained evidence of a second response in stimulating nerves with induction shocks. The shocks employed by them were presumably of longer duration than those from the coreless Porter coil of our experiments. A second cathodal response may have been obtained if the effective duration of their shock was longer than the duration of the absolute refractory period of the nerve but more probably their second response was anodal in origin. Our experiments demonstrate that with short induction shocks the cathodal response is never repetitive. It is impossible to produce a Pflüger's or closing tetanus but, as has been shown, a Ritter's or opening tetanus is obtainable.

On first thought the order of stimulation in nerve with short induction shocks seems similar to that demanded by the law of stimulation of Pflüger. The law as formulated by him was the result of observations made with galvanic stimuli of considerable duration applied to a muscle-nerve preparation. For weak and moderate currents the results with galvanic stimulation and induction shocks are similar for reasons now not difficult to understand. With strong galvanic stimulation the failure to obtain a response at the make of an ascending current and the break of the descend-

ing current in a nerve-muscle preparation is due to the electrotonic depression under the electrode proximal to the muscle blocking the response from the electrode distal to the muscle. With ordinary short induction shocks the failure to obtain a response from the electrode distal to the muscle is due to the blocking of the impulse from this electrode by the impulse from the proximal electrode travelling in both directions in the same nerve fibers. Complete blocking of the processes from one electrode is only possible when the cathode is distal because its response is never repetitive. When the anode is distal repetitive responses can still be effective even though the first response at the anode is blocked. Electrotonic depression from maximal or reasonably supramaximal induction shocks is not adequate to actually block conduction in a nerve. For this reason the blocking of one wave by the other differs from Pflüger's third case where the blocking is purely electrotonic. In galvanic current stimulation the action potential waves do not block each other owing to the long interval between the make and break usually obtaining in the demonstration of Pflüger's law.

## SUMMARY

Direct evidence of stimulation at the anode with short induction shocks is presented. In irritable nerve the response at the anode may be repetitive (Ritter's opening tetanus), the number of responses varying with the irritability of the nerve.

The recorded cathodal and anodal responses arise in different fibers with stimuli below the maximal for the cathodal response. The response arising under the stimulating electrode proximal to the leads of the recording apparatus travelling in both directions blocks the first response from the more distal electrode when the former stimulates all the fibers. Repetitive anodal responses are not blocked by a proximal cathode after the cathodal response has passed the anode.

Artefacts and the separation out of waves on conduction are not responsible for the multiple waves described here.

The similarity of the order of stimulation in nerve with increasing strengths of short induction shocks to the order of stimulation by galvanic currents as outlined by Pflüger for stages I and II is shown. A difference arises in stage III where with Pflüger's galvanic currents of adequate duration the fiber blocking is purely electrotonic and not the result of interference of processes travelling in opposite directions.

An induction shock is similar in its stimulating action to a galvanic current of short duration. It differs from a galvanic current of longer duration in not giving rise to repetitive responses at the cathode and in producing a much less marked degree of electrotonic depression.

I wish to acknowledge the critical suggestions of Dr. George H. Bishop in this work.

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# THE HARMONIC ANALYSIS OF INTRAVENTRICULAR PRESSURE CURVES

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Received for publication June 30, 1928

Investigators who have devised practical forms of manometers recording by optical registration have experienced considerable difficulty in selecting the constants so that an adequate natural frequency and a sufficient degree of sensitivity exist at the same time. Frank's equation $T = 2\pi\sqrt{\frac{M'}{E'}}$ makes it clear why this is so. The natural period, $T$, is decreased by reducing the effective mass, $M'$, or by increasing the volume elasticity coefficient, $E'$. There are practical limits, however, beyond which the effective mass cannot be reduced and yet retain a practical form of instrument. There is likewise a limit beyond which $E'$ cannot be increased without reducing the ratio between pressure changes in the cardiovascular system and amplitude of record, i.e., the sensitivity. Adequate amplitude is, however, almost as important as accurate amplitude and contour; without it, smaller oscillations or bends are often difficult to locate with precision, and fine vibrations are entirely obscured when pressure gradients are steep.

In membrane manometers, the inverse relation between natural frequency and sensitivity is easily altered by changing the thickness and tension of the rubber membrane. This is illustrated by the following data derived from tests with an optical manometer devised by the author:

In cardiodynamic researches reported at various times, the writer has taken care that manometers with all three of these ratings were employed in different experiments of the same series, and, in this way, curves recorded with different ordinate values were at his disposal. Most of the published intraventricular pressure curves were obtained with manometers having a natural frequency of about 180, and a sensitivity ratio of 3.5:10; and, as a matter of fact, they have proved the most valuable for analysis. In the study of right ventricular and pulmonary pressures, the most satisfactory curves were obtained by manometers whose sensitivity was 6:10, and whose natural frequency was about 150 per second.

It becomes desirable, however, to reinvestigate the adequacy of

manometers with such lower frequencies when employed for registration of intraventricular pressures and to establish definitely the experimental limitations to their use. It seems particularly appropriate that the writer should do so inasmuch as Straub (1914) has questioned the validity of some of his work on the lesser circulation, holding that the frequency of the manometers used (157 per sec.) was inadequate for such studies, and in later communications (1923) has stated it as his belief that natural frequencies of 200 and 300 per second are minimal for manometers used to record right and left intraventricular pressures, respectively.

*Previous methods for determining the minimal requisite natural frequency.* The minimal frequency required of a manometer in order to reproduce the phasic and amplitude relations of pressure fluctuations is not a matter of authoritative opinion; it must be established by actual physical tests. The methods adopted in the past have been laborious and not entirely satisfactory. Briefly reviewed, the following procedures have been adopted:—Curves were recorded by manometers having different natural frequencies, i.e., ranging from the highest attainable to those of a lower

TABLE 1

| SENSITIVITY | NATURAL FREQUENCY |
|---|---|
| 2:10 | 233 |
| 3.6:10 | 181 |
| 6:10 | 150 |

order. By employing somewhat complicated corrective procedures, described at various times by Mach (1863), Einthoven (1894, 1895, 1903a, 1903b, 1906), Frank (1903b, 1905), and Garten (1902), the true contour was established. The lowest natural frequency at which such a manometer reproduces a curve that demands no essential correction is assumed to be the lowest permissible frequency for the experimental conditions obtaining. Using this method, Frank (1905) corrected many arterial pressure curves recorded by manometers with different natural frequencies and concluded that a frequency of 104 is almost sufficient to register the central arterial pulse correctly. In his later development of the mirror manometer (1910) with a natural frequency of 180 and of his optical spring manometer with a natural frequency of 300, he believes he has devised instruments which far exceed all requisites of pressure registration.

Whether or not manometers used to record intraventricular pressures should have a higher or lower minimal frequency than those designed for recording arterial pressures may be disputed on *a priori* grounds, but no final decision can be rendered in this manner. It may be argued with Straub (1923) that since the gradient of the intraventricular pressure

curve is steeper and the amplitude much larger, a higher frequency is demanded. But it may also be pointed out that the arterial pressure curves contain many rapid adventitious vibrations which appear not to exist in the ventricles. Hence, it may be argued with equal validity that a manometer which is capable of reproducing all of these minor deflections with accuracy may also be trusted to reproduce the much smoother type of pressure fluctuation exhibited in the ventricular curve. At various times during the last 15 years, the writer has attempted to determine the minimal natural frequency required to record intraventricular pressure curves accurately. These efforts consisted in attempts to determine whether curves recorded by manometers having natural frequencies over 200 per second were more accurate than those obtained by instruments whose frequency was about 150 per second. The results were far from conclusive. At one time the corrective procedures suggested by Frank (1903, 1905) were applied to ventricular pressure curves recorded by manometers of essentially different frequencies. The method proved difficult in its application to the rounded ventricular pressure curves, and the results were not convincing. Attempts were also made to compare identical ventricular pressure curves recorded with manometers of different frequencies simultaneously or in rapid succession. Both procedures are subject to uncontrollable experimental errors. When more than one manometer cannula is inserted into a ventricle, the probability that one or both are blocked or pocketed during certain phases of ventricular systole is very great. Consequently, curves were obtained whose contours varied so strikingly that the differences could not easily be attributed to the manometers. The most consistent results were obtained by leaving a single manometer in position and changing only the recording capsules, but as this introduced a delay, circulatory conditions often changed somewhat and the curves were not strictly similar on this account. In only a few experiments was there reason to believe that the curves were similar, but the fact was difficult to establish because the amplitudes were so different. Until some convenient and reliable mechanical method is devised for altering the ordinate values of pressure curves without changing the abscissal values, there will be great difficulty in proving that two curves of differing amplitudes do or do not correspond in every detail.

There is, however, a physical method by means of which the question as to the minimal frequency required to reproduce intraventricular curves accurately can be settled. Theoretical and mathematical analyses of Frank (1911) and those of Broemser (1914, 1918) which supplement them have clearly proven that curves are accurately reproduced by a manometer—i.e., within the limitations of analysis or calculation—provided the natural frequency is at least 4 or 5 times greater than the shortest sig-

nificant component entering into the true curve.[1] Obviously, however, such a criterion is not particularly useful unless the shortest significant component is known. Now, the pressure curves from the cavities of the heart and from the large vessels like any periodic compound curve may be resolved by the method of harmonic analysis based on Fourier's theorem into its simple harmonic components. If such a curve is analyzable into a sufficient number of components, the shortest significant one can be established and the minimal frequency required of a manometer can be precisely determined. Broemser (1918) attempted such an analysis of the arterial pressure curve of a rat, but the harmonic analyzer at his disposal was unfortunately limited to the reproduction of six components. He found this number inadequate for the resynthesis of a curve corresponding in form and amplitude to that of the original. The number of components required to do this could not be actually determined owing to the limitations of the analyzer, but he ventured the opinion that at least 30 to 50 appeared to be necessary.

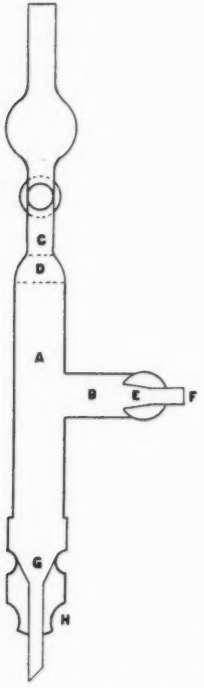


Fig. 1. Lateral sectional view of optical membrane manometer. Dimensions and constants given in text.

Since Prof. Dayton Miller of Case School of Applied Science has developed both a Henrici harmonic analyzer and synthesizer extended to thirty components (1916), and graciously offered to analyze a series of records for me, it appeared desirable to determine whether the smallest significant component entering into the intraventricular pressure curve could be determined in this way.

*Apparatus and experimental procedure.* The intraventricular pressure curves used for analysis were recorded by the universal optical manometer described in preliminary form by the writer and Baker (1924). As the physical constants of this apparatus are of importance in analyses of this sort, they may be noted briefly in relation to the drawing of figure 1. Vertical tube, *A*, length, 8 cm.; internal diameter, 1.3 cm. Side tube *B*, length, 2.5 cm.; internal diameter, 1.3 cm. Tube *C*, length up to stopcock, 2 cm.; diameter, 0.6 cm. Height of glass cupola *D*, 0.6 cm. Con-

[1] It is interesting to note that a similar multiple of the lowest component entering into electrocardiograms was independently established by Fahr (1914) as the minimal required frequency of the string galvanometer.

ical passage in ball $E$, length, 1 cm.; diameters of openings, 0.9 and 0.5 cm. Cylindrical passage $F$, terminating in segment opening, length, 0.7 cm.; diameter, 0.4 cm. Conical connection with cannula $G$, length, 0.7 cm.; diameters of openings, 1.3 and 0.3 cm. Cylindrical passage through stopcock and cannula $H$, diameter, 0.3 cm.; length, 3.3 cm. for straight cannula and 4.5 cm. for curved arterial cannula. Diameter of segment capsule, 0.4 cm.

The effective masses $M'$ of the several portions calculated according to Frank's formula are as follows: $A = 6.0$; $B = 1.8$; $C = 7.9$; $D = 0.25$;

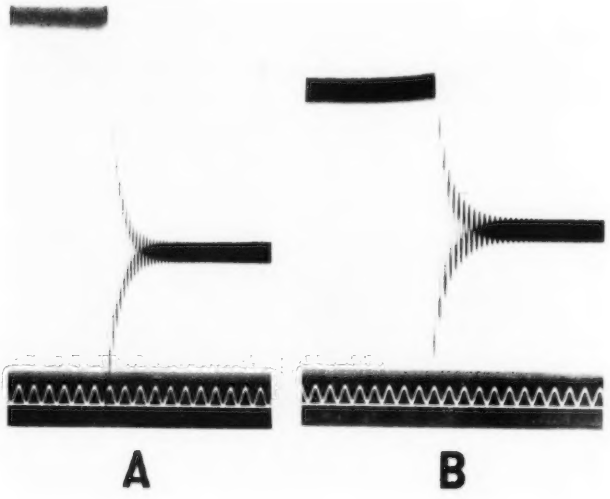


Fig. 2. Vibration frequency and damping tests made with author's universal manometer covered with membranes of different thicknesses. Curve $A$, sensitivity, 2:10; $N = 233$ per second. Curve $B$, sensitivity, 6:10; $N = 150$. For other data cf. table 2. Tuning fork, 50 per second (expt. C-401).

$E = 0.7$; $F = 5.5$; $G = 0.6$; $H = 4.7$ (straight cannula), and 6.3 (curved cannula). Total effective mass, 27.45 (straight cannula), 29.05 (curved cannula).

The vibration frequency of the manometer fitted with three different thicknesses of rubber, the logarithmic decrement, and damping factor, $D$, as defined by Frank (1903A) were determined experimentally by an adaptation of the method of Frank, i.e., by allowing the cannula to dip into a small chamber covered with highly stretched thin rubber, placing the entire system under static pressure, and suddenly bursting the elastic membrane by a hot point. Two records of such tests are shown in figure 2.

The periods of the double vibrations $T$ and the natural frequency ($N = 1/T$) are easily calculated by reference to the tuning fork below. The ratio by which the successive single vibrations decrease is the decrement

TABLE 2

| DATA FROM FIGURE 2A | | | | DATA FROM FIGURE 2B | | | |
|---|---|---|---|---|---|---|---|
| Vibration number | Amplitude | Natural logarithm | Logarithmic decrement | Vibration number | Amplitude | Natural logarithm | Logarithmic decrement |
| 1 | 65 | 4.17439 | | 1 | 72 | 4.24850 | |
| 2 | 55 | 4.00733 | 0.16706 | 2 | 63 | 4.11087 | 0.13763 |
| 3 | 47 | 3.85015 | 0.15718 | 3 | 55 | 3.97027 | 0.14060 |
| 4 | 40.8 | 3.71357 | 0.13658 | 4 | 48 | 3.82864 | 0.14163 |
| 5 | 35 | 3.55535 | 0.15822 | 5 | 44 | 3.68888 | 0.13976 |
| 6 | 30.5 | 3.41743 | 0.13792 | 6 | 37 | 3.55533 | 0.13355 |
| Average | | | 0.151+ | | | | 0.1385 |
| $D$ = 0.445 | | | | | 0.490 | | |

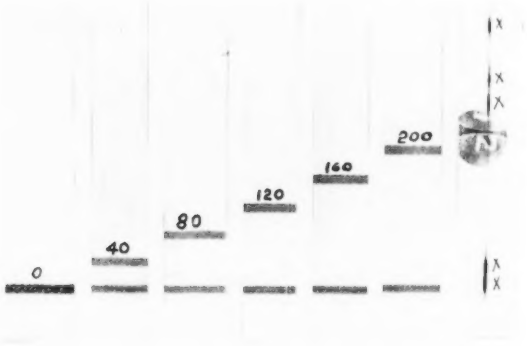


Fig. 3. Six segments of records showing good proportionality of deflection upon standardization of optical manometer from 0 to 200 mm. Lower line, base line. Last segment shows at $X$ tests for absence of parallax of several manometers. Note precise vertical alignment. (About $\frac{1}{2}$ actual size.) (Expt. C-407-XII.)

and the natural logarithm is the logarithmic decrement. The damping coefficient $D$, is determined by Frank's equation

$$D = \frac{\lambda}{\sqrt{\pi^2 + \lambda^2}}$$

in which $\lambda$ represents the logarithmic decrement. The reliability of the procedure is attested by the close correspondence between the logarithmic decrement of succeeding vibrations which together with other data are given in table 2.

The sensitivity of the manometer was determined by calibration under static conditions and is expressed as a ratio of the deviations of the light beam on the photokymograph 1 meter distant to the pressure increase, both expressed in millimeters. The excellent proportionality between equal pressure increments and recorded deflections is shown in the curve

TABLE 3

| COMPONENT | CURVE 1 | | CURVE 2 | | CURVE 3 | | CURVE 4 | | CURVE 5 | | CURVE 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amplitude | Phase | Amplitude | Phase | Amplitude | Phase | Amplitude | Phase | Amplitude | Phase | Amplitude | Phase |
| | *mm.* | *degrees* | *mm.* | *degrees* | *mm.* | *degrees* | *mm.* | *degrees* | *mm.* | *degrees* | *mm.* | *degrees* |
| 1 | 125.1 | 287 | 152.8 | 289 | 34.4 | 293 | 137.2 | 293 | 183.9 | 267 | 125.2 | 298 |
| 2 | 6.1 | 211 | 36.6 | 284 | 5.6 | 268 | 12.6 | 311 | 20.5 | 296 | 14.5 | 325 |
| 3 | 16.1 | 161 | 18.6 | 156 | 2.3 | 177 | 18.8 | 165 | 29.5 | 124 | 18.1 | 203 |
| 4 | 2.5 | 18 | 11.8 | 148 | 1.1 | 148 | 8.5 | 190 | 7.1 | 67 | 7.4 | 217 |
| 5 | 4.3 | 43 | 1.6 | 86 | | | 4.9 | 22 | 9.9 | 343 | 5.9 | 106 |
| 6 | 1.3 | 214 | 4.6 | 21 | | | 3.4 | 64 | 1.8 | 340 | 2.2 | 144 |
| 7 | 1.8 | 269 | 2.1 | 351 | | | 1.5 | 236 | 5.1 | 206 | 2.5 | 8 |
| 8 | 0.6 | 90 | 1.5 | 244 | | | 2.3 | 269 | 1.9 | 158 | 1.4 | 71 |
| 9 | 0.2 | 146 | 1.7 | 180 | | | 0.8 | 144 | 2.9 | 30 | 1.7 | 270 |
| 10 | 0.2 | 255 | 0.8 | 214 | | | 1.6 | 134 | 1.8 | 330 | 0.8 | 319 |
| 11 | | | 0.1 | 199 | | | 0.4 | 197 | 2.1 | 244 | 0.9 | 171 |
| 12 | | | 0.6 | 266 | | | 0.9 | 351 | 0.6 | 177 | 0.5 | 175 |
| 13 | | | 0.7 | 251 | | | 0.8 | 317 | 0.7 | 79 | 0.9 | 51 |
| 14 | | | 0.3 | 140 | | | 0.2 | 103 | 0.3 | 290 | 0.2 | 129 |
| 15 | | | 0.3 | 92 | | | 0.7 | 104 | 1.0 | 310 | 0.4 | 290 |
| 16 | | | 0.2 | 28 | | | 0.3 | 309 | 0.2 | 259 | 0.3 | 352 |
| 17 | | | 0.7 | 158 | | | 0.5 | 144 | 0.6 | 164 | 0.7 | 139 |
| 18 | | | 0.2 | 188 | | | 0.6 | 168 | 0.0 | 55 | 0.6 | 193 |
| 19 | | | 0.2 | 114 | | | 0.2 | 144 | 0.5 | 84 | 0.3 | 26 |
| 20 | | | 0.1 | 99 | | | 0.4 | 16 | 0.3 | 147 | 0.4 | 80 |
| 21 | | | | | | | 0.1 | 98 | | | | |
| 22 | | | | | | | 0.3 | 10 | | | | |
| 23 | | | | | | | 0.3 | 249 | | | | |
| 24 | | | | | | | 0.3 | 253 | | | | |
| 25 | | | | | | | 0.1 | 128 | | | | |
| 26 | | | | | | | 0.3 | 173 | | | | |
| 27 | | | | | | | 0.2 | 205 | | | | |
| 28 | | | | | | | 0.4 | 69 | | | | |
| 29 | | | | | | | 0.1 | 90 | | | | |
| 30 | | | | | | | 0.2 | 223 | | | | |

of figure 3. Incidentally this shows that by selecting a good grade of rubber and stretching it tightly over the manometer capsules, the diminishing extension supposed to exist in a membrane manometer is reduced to an immeasurable degree within the pressure ranges used.

At a sensitivity ratio of 2:10, and with a vibration frequency of 233 per

second (fig. 1A), the volume-elasticity coefficient $E'$ (calculated from formula $T = 2\pi\sqrt{\frac{M'}{E'}}$) is 58,982 $\times$ $10^3$, and, at a sensitivity ratio of 6:10 and a frequency of 150 per second (fig. 1B), is 24,304 $\times$ $10^3$.

*Analysis of records.* Six typical intraventricular pressure curves were selected for study. These curves were projected by a reflectoscope system so that they were enlarged exactly to a standard length of 400 mm., a requirement for use in the Henrici harmonic analyzer. I am indebted to Professor Dayton Miller of Case School of Applied Science for the harmonic analysis of the curves as well as for the measurement of the amplitudes, phase angles, etc., of successive components.

RESULTS. The data of table 3 give a general survey of the results. It is apparent at a glance that in every case the amplitudes of components at or near the tenth become so small that they barely exceed the width of a line. When it is recalled that the redrawn curves used for analysis were enlarged approximately 5 times, we see that their amplitude is a matter of hundredths of a millimeter in the original curve. We may at once draw the probable conclusion that the smallest significant component lies somewhere between the 8th and 12th in the average curve. If this be true it should be possible to resynthesize the original curve by using such a number of components. This was done in all six experiments with the result that remarkably close correspondence with the original curves was obtained by using 10, 9, 4, 10, 11 and 9 components respectively. The knowledge that a far smaller number of significant components enters into the ventricular pressure curve than was anticipated may be of service to others who may be limited to the use of analyzers for a smaller number of components. Attempts were also made to resynthesize curves utilizing a greater number of components. Such curves, to our surprise, deviated more from the original than those synthesized from a judiciously chosen number of significant components. Whether this is the result of technical limitations in dealing with components of such small amplitude or is due to the existence of additional higher components of exceedingly small amplitude which are capable of neutralizing the unnatural deviations introduced cannot be stated. The fact remains that, in practice, curves resynthesized from approximately 10 components show the closest degree of superposition.

Two sample curves are discussed in greater detail in order that the reader may obtain a more accurate idea as to the magnitude of the deviation between the recorded and resynthesized curves. This cannot easily be done by superimposed curves, for the correspondence is so exact that differences tend to vanish when the curves are reduced to a size suitable for illustration. Consequently other devices must be resorted to. The illustration in figure 4 shows an original left ventricular pressure curve

with a dome-shaped summit (curve 4). This was taken with a manometer having a frequency of 233 per second and factors given in table 2.

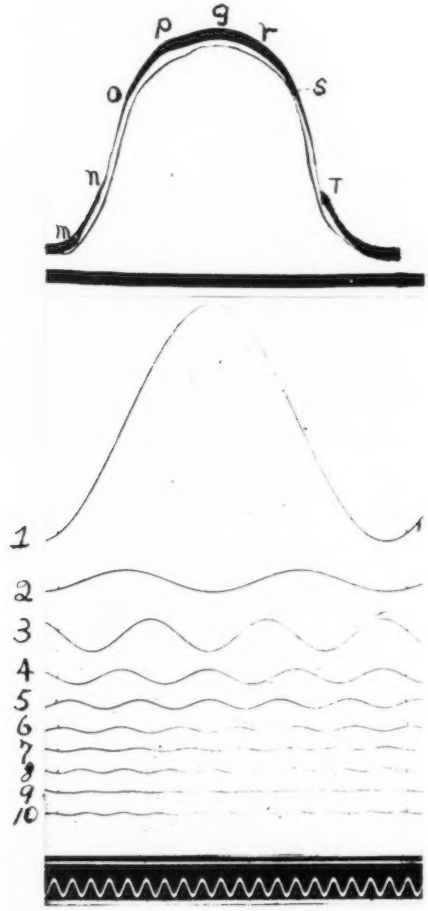


Fig. 4. Original intraventricular pressure curve, above; resynthesized curve, just below. Curves 1–10 first 10 components. Time, 0.02 second (expt. C-338-I).

Underneath, the first ten components are correctly redrawn as to phase and amplitude, and just beneath the original pressure record the curve resynthesized from the 10 components is drawn. These line curves were

obtained by rephotographing the original larger chart of components to such a size that the synthesized curve is a trifle smaller than the original

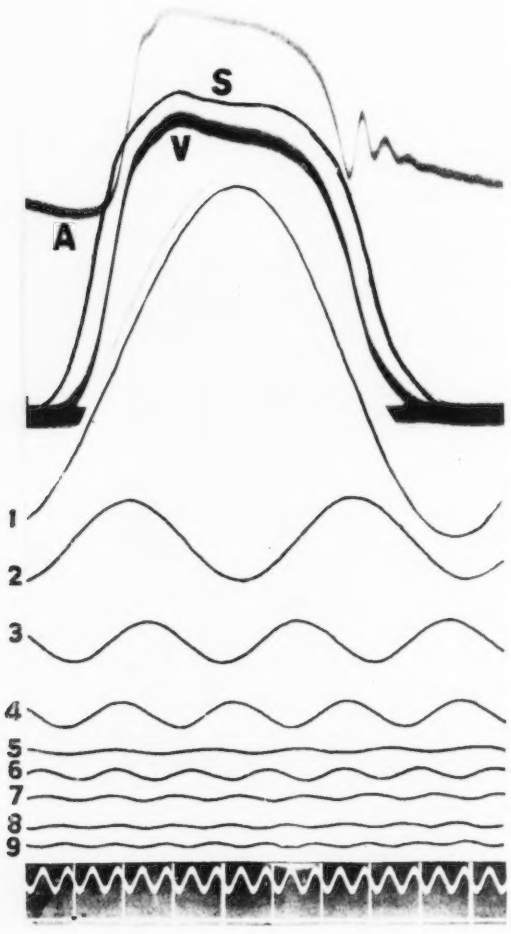


Fig. 5. Ventricular pressure curve, *V;* aortic pressure curve, *A* resynthesized curve, *S*. Curves 1–9, first 9 components. Time 0.02 second. (Expt. C-361-II.)

and hence fits beneath the recorded pressure curve as shown in figure 4. Such comparisons show: 1, that the original curve is correctly reproduced in amplitude, i.e., within limitations of measurement; and 2, that the chief

bends in the curve, e.g., at $m$, $n$, $o$, $p$, $q$, $r$, $s$, $t$, $u$ are reproduced by the number of components. The deviations consist of a slight difference in the gradient of the initial slow rise, $m - n$; a slight difference in the prominence of the humps, $p$ and $q$, and a failure to reproduce the smaller variations beyond $t$.

Figure 5 similarly shows original aortic and ventricular pressure curves (curve 2). The latter is of the type described as having a plateau summit. Below are a series of 9 components and drawn over the original is a curve resynthesized from them. These curves were rephotographed from the large original to such a size that the resynthesized curve is a trifle larger than the original and hence fits just over the latter in the illustration. On superposition, the amplitudes were found to correspond except that the enlarged original was about 2 mm. higher. The exact correspondence of the details in the two curves is evident from the records. This precise reproduction of the original pressure curve from synthetic analysis of 9

TABLE 4

| HEART RATE | DURATION OF ENTIRE CURVE | T. OF 10TH COMPONENT | N. OF 10TH COMPONENT | MINIMAL VIBRATION FREQUENCY (= 5 N) |
|---|---|---|---|---|
| 140 | 0.32 | 0.032 | 31.2 | 156.0 |
| 152 | 0.291 | 0.029 | 34.4 | 172.0 |
| 171 | 0.28 | 0.028 | 35.9 | 179.5 |
| 200 | 0.26 | 0.026 | 39.0 | 195.0 |
| 229 | 0.252 | 0.025 | 40.0 | 200.0 |
| 240 | 0.240 | 0.024 | 41.6 | 208.0 |
| 260 | 0.23 | 0.023 | 43.0 | 215.0 |

components is of especial interest as it was recorded by a manometer having a natural frequency of only 150 per second, and constants given in table 2.

There can be no doubt that ordinarily curves of normal contour can be reconstructed from the first ten components into which the originals are resolved by harmonic analysis. If we accept the theoretical analyses of Broemser (1918) we may further conclude that a manometer having a frequency five times as great as the tenth component reproduces records that are accurate as to amplitude, phasic relations, and contour, within the limits of measurement.

The value of the 10th component obviously depends on the heart rate. In the record reproduced in figure 4, the period of the entire wave was 0.400 second and in the record of figure 5, 0.380 second, making the 10th component roughly 0.04 second. Eliminating diastolic pauses, the frequency of these 10th components thus becomes 25 per second at heart rates of 110 and 130 per minute, respectively. It is obvious that up to

these rates as accurate curves would have been recorded by a manometer with only 125 vibrations per second, and that the frequencies of the manometer employed were definitely in excess of that required in both cases. It is obvious, however, that, as the duration of the original pressure curve decreases with progressive acceleration of the heart, the value of the tenth component must also diminish and the minimal frequency requirements of a manometer must become greater. In table 4 are presented data from experiment C387 showing such calculations at heart rates from 140 to 260 per minute, the latter being about the highest speed at which the ventricle is capable of responding to supra-ventricular stimuli. The results show clearly that, according to these standards, a manometer with a natural frequency of 150 may not be used above heart rates of 140

TABLE 5

| COMPONENT | $N_c$ | PHASE | AMPLITUDE | WITH MANOMETER $N_a = 233$ $R_n = \frac{N_c}{N_a}$ $D = 0.445$ | | | WITH MANOMETER $N_a = 150$ $R_n = \frac{N_c}{N_a}$ $D = 0.490$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $R_n$ | Phase correction | Amplitude correction factor | $R_n$ | Phase correction | Amplitude correction factor |
| | | *degrees* | *mm.* | | *degrees* | | | *degrees* | |
| 1 | 2.5 | 293 | 137.2 | 0.0107 | −0.52 | 1.0001 | 0.0166 | −0.9 | 1.0002 |
| 2 | 5.0 | 311 | 12.6 | 0.0214 | −1.08 | 1.0004 | 0.0232 | −1.33 | 1.0005 |
| 3 | 7.5 | 165 | 18.8 | 0.0322 | +1.58 | 1.0010 | 0.0498 | +2.8 | 1.0010 |
| 4 | 10.0 | 190 | 8.5 | 0.0428 | −2.16 | 1.0013 | 0.0664 | −3.9 | 1.0024 |
| 5 | 12.5 | 22 | 4.9 | 0.0535 | +2.7 | 1.0018 | 0.0830 | +4.66 | 1.0034 |
| 6 | 15.0 | 64 | 3.4 | 0.0643 | +3.25 | 1.0027 | 0.0996 | +5.6 | 1.0057 |
| 7 | 17.5 | 236 | 1.5 | 0.0749 | −3.83 | 1.0034 | 0.1162 | −6.5 | 1.0059 |
| 8 | 20.0 | 269 | 2.3 | 0.0858 | −4.5 | 1.0044 | 0.1328 | −7.5 | 1.0063 |
| 9 | 22.5 | 144 | 0.8 | 0.0963 | +4.9 | 1.0057 | 0.1492 | +8.0 | 1.0089 |
| 10 | 25.0 | 134 | 1.6 | 0.1070 | +5.5 | 1.0061 | 0.1660 | +9.33 | 1.0092 |

per minute, and one with a frequency of 180 up to heart rates of about 171 per minute. These calculations assume, however, that all of these curves can be analyzed as far as 10 significant components. This appears not to be the case. Upon harmonic analysis, it is found that, as the rate increases and the curves become smoother in form and smaller in amplitude, they can usually be resynthesized by a progressively smaller number of components. The analysis of curve 3 shown in table 3 is a case in point. It corresponds to one shown as occurring at a heart rate of 260 in table 4. Analysis could not easily be extended beyond the fourth component and perfect superposition on the original resulted when a curve was resynthesized by using these four components alone. Since the fourth, the last significant component, had a period of 0.075 second and a frequency of

roughly 18, a manometer with a frequency of only $5 \times 18$ or 90 per second would seem to be adequate.

While such curves may be considered to be accurate within the limits of physical measurement, it does not follow, of course, that the intraventricular pressure fluctuations are reproduced with absolute precision. The degree and nature of the distortion which still exists as to amplitude and contour can, however, be accurately determined according to the equations evolved by Broemser (1914, 1918). To do this, it is necessary first, to establish the natural frequency, $Na$, the logarithmic decrement ($\lambda$) and damping coefficient ($D = \frac{\lambda}{\sqrt{\pi^2 + \lambda^2}}$) of the manometer.

After a pressure curve has been harmonically analyzed it is necessary to calculate for each component (1) the frequency ($N_c$): (2) the ratio $\frac{N_c}{N_a}$, conveniently designated $R_n$, (3) the amplitude in millimeters and (4) the phase angle in degrees and decimals of a degree. The spatial phase correction ($\phi$) can then be determined for each component according to the formula

$$\tan \phi = \frac{2 D R_n}{R_n^2 - 1}$$

$\phi$, so calculated, expresses the angle in decimals of a degree which must be added to the several components if the angle lies between 0 and 180°, and subtracted if it lies between 180° and 360°.

Corrections for errors in amplitude are made by multiplying the amplitude of each component by a correction factor, $Q$, calculated according to the equation:

$$Q = \frac{1}{\sqrt{(1 - R_n^2)^2 + 4 D^2 R_n^2}}$$

Having corrected each component in this way as to phase, angle, and amplitude, another curve can be resynthesized by using these corrected values.

In table 5, results are presented showing the corrections that would be effective for ten components in the case of the curve analyzed in figure 4, assuming that manometers with frequencies of 233 and 150 respectively had been employed. It is only necessary to scan the figures in the 7th and 10th columns showing the factor by which the amplitude must be multiplied in order to realize the exactness with which the amplitude of the original curve is reproduced. Likewise, a comparison of columns 6 and 9 clearly shows that the phasic displacement particularly of the larger components is so small that no measurable lag in the rise of the original

curve could have existed. In fact, the variations seemed so insignificant as to make it of no value to attempt resynthesis of the corrected curve. Even the maximum correction in the case of the very small 9th and 10th component is very small and the difference between the two manometers is less than 4 degrees.

## SUMMARY

1. Intraventricular pressure curves were recorded by an optical manometer ranging in frequencies from 233 to 150 per second, and whose physical constants are presented. These curves were accurately enlarged, redrawn, and analyzed by means of Miller's harmonic analyzer for 30 components. Subsequently, curves were resynthesized from the components so established.

2. As the 10th component of the enlarged curve was less than 1 mm., it was considered probable that this represents, approximately, the shortest significant component. This deduction was corroborated by the fact that curves practically reduplicating the original pressure curve could be resynthesized by using the first 9 or 10 components only.

3. Following the analysis of Broemser that a manometer which has a frequency fivefold that of the shortest significant component (in these cases approximately the 10th), it was established that a manometer with a natural frequency of 125 should be capable of recording accurately the ventricular pressure curves from a dog up to heart rates of about 130 per minute, one with a frequency of 150 up to a heart rate of 140, one with a frequency of 180 practically up to the limits of speed with which the ventricle is capable of responding to supraventricular stimuli.

4. Broemser's formulae by which the phase angle and amplitude of each component may be corrected was applied, and the calculations showed that ventricular pressure curves recorded by manometers with natural frequencies of 150 to 233 per second require only insignificant corrections, i.e., such as are not capable of physical measurement.

5. The conclusion is reached that the natural frequency required to record intraventricular pressure curves accurately is much lower than has often been surmised. This fact is of practical importance as it is not necessary to sacrifice sensitivity to low natural period in the use of such recording instruments.

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# A COMPARATIVE STUDY OF THE ACID-BASE EQUILIBRIUM OF BILATERALLY NEPHRECTOMIZED AND ADRENALECTOMIZED DOGS

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Received for publication June 5, 1928

For several years the writer (1927) and his students have been studying the effect of bilateral adrenal extirpation in cats and dogs, and reached the conclusion that one of the train of causes resulting in death from adrenal ablation is acid intoxication. As a result of our experiments the hypothesis was advanced that the adrenal cortex secretes a hormone which in some manner assists in maintaining the normal functioning of the kidney. We were interested in the fact that the type of acidosis which appears during adrenal insufficiency is similar to that occurring in uremia and the experimental nephritis produced by nephrotoxic substances such as uranium nitrate. As a further means of testing the idea whether or not the kidney is involved in adrenal insufficiency, the writer undertook to make a careful comparison of the symptoms and blood findings occurring in adrenal insufficiency and those which follow kidney extirpation. For details regarding adrenalectomy and the blood changes following the onset of adrenal symptoms reference is made to the previous publications from this laboratory on the subject. In the present paper only data pertaining to renal insufficiency will be set down in detail.

Clinicians and pathological chemists have been aware for some years that the terminal stages of certain types of nephritis are complicated by a well-marked acid intoxication. In fact uremic symptoms are practically if not always accompanied by chemical changes in the blood indicative of acidosis. The acidosis is very probably not the cause of uremia but is associated with it. The blood of uremic individuals with acid intoxication shows lowered $CO_2$ capacity, $CO_2$ content and pH, and increase in phosphate and sulphate ions and non-protein nitrogen. The change in the acid-base equilibrium may be so great as to be the actual cause of death in some cases, according to Chase and Myers (1920).

The assumption has been that the acidosis of renal insufficiency is primarily due to retention of the acids formed in the course of normal metabolism due to inability of the injured kidney to excrete them.

However, it is not at all clear that total cessation of kidney functioning

results in acid intoxication. Thus Ptaszek (1927) recently reported that he was unable to obtain very marked symptoms of acid intoxication in dogs surviving 2 to 5 days after double nephrectomy. There was a small diminution in alkali reserve but the slight decrease was in marked contrast to the fall observed in cases of human uremia. He expresses the opinion that when the kidneys are extirpated some extra renal mechanism maintains the alkali reserve approximately normal, whereas in uremia the extra-renal mechanism breaks down along with the kidney.

Andrews (1927) reports several cases of dogs surviving 4 to 10 days following total nephrectomy. The animals did not show evidences of acid intoxication even in the terminal stages. They were active, played about and had good appetites.

While the writer is not directly concerned in the present pages with the problem of uremia, nevertheless some of the data suggest that the primary cause of uremia, is probably extra-renal in origin and that possibly the adrenal glands, and especially that portion known as the cortex, may be involved.

BILATERAL NEPHRECTOMY. Large, well-nourished dogs, averaging in weight between 14 to 20 kilos, were employed as experimental animals. It has been our experience that large animals survive bilateral nephrectomy longer than the smaller dogs. They were kept in the laboratory for several days before using in order to accustom them to their new surroundings. They were fed cooked meat scraps, bread and occasionally a half pint of milk. The right kidney was removed first through an incision nearly parallel with the last rib. The operation is not severe and can be accomplished with practically no bleeding, the animal rapidly recovers and is normal, after 5 to 10 days the left kidney was extirpated in a similar fashion. Such operated dogs behave in normal fashion for two and sometimes three days. They are lively, eat and drink and walk about after the operation.

Several days following unilateral nephrectomy the animals were bled from the heart and from 60 to 70 cc. of arterial blood taken for chemical examination. Determinations were made of the $CO_2$ capacity, $CO_2$ content, pH, inorganic sulphate and phosphate, sugar, chlorides and urea. The methods employed were as follows: $CO_2$ capacity and content, Van Slyke (1927), pH, Myers, Schmitz and Booher (1923), using Myers and Muntwyler's correction for dog plasma (1927), sulphate, Denis (1927); phosphate, Benedict and Theis (1924); sugar, Folin (1922); chlorides, Whitehorn (1920); urea, Myers, (1924).

Following removal of the remaining kidney the animals were again bled when marked symptoms developed, but in all cases the determinations were made, while the animal was in good condition and before it became moribund. In several animals three determinations were made, the final

blood being taken just before the pre-mortal coma set in. It was found impracticable to bleed the animals frequently after the onset of symptoms since a considerable quantity of blood was required for the analysis. Too frequent bleeding weakens the animals and greatly shortend the life span of the nephrectomized dogs. Generally bilaterally nephrectomized dogs are normal for about 40 to 50 hours after the second operation, and occasionally are normal for three days. They eat, walk about and seem in excellent condition. The first symptoms are malaise and loss of appetite. The dogs sniff at proffered food and may eat a bite or two and then turn away. They appear listless, drink considerable water and lie quiet for hours at a time, but when aroused, will walk about in normal fashion. Vomiting is generally a prominent symptom, especially on the third day after the operation. The dogs vomit large quantities of clear fluid and mucus. If fed milk by the stomach tube it is usually vomited within a short time. One of the animals did not vomit at any time during the five days it survived double nephrectomy, and another animal vomited but twice during a four day survival period. In general however, vomiting is a prominent symptom. As symptoms progress, the animal shows marked weakness of the hind legs and sways from side to side when walking. In the terminal stage the animal passes into coma and dies. Fifteen dogs were used in the work. Two animals slipped ligatures within forty hours after the second operation and died of hemorrhages. Three dogs died during the night before the second blood was obtained for analysis, one had survived the second operation 74 hours, one 81 hours and the third 93 hours, and one animal developed pneumonia after the second operation and was not used.

In table 1 are presented the data from eight of the double nephrectomized animals.

An analysis of the data presented in table 1 reveals some interesting facts.

It seems quite evident that acid intoxication is not only not a prominent feature of the syndrome following bilateral nephrectomy but is entirely absent from most cases, and only doubtfully present in a few individuals.

It is somewhat surprising to find that total loss of renal function is not associated with acidosis. In uremia, acidosis is almost invariably present and in some cases, according to Myers and Chase, is of sufficient magnitude as to probably be the actual cause of death.

The assumption has commonly been made that the acidosis observed in uremia and that resulting from nephrotoxic substances such as uranium nitrate, is probably due to renal insufficiency with resultant piling up in the blood of acid metabolites normally excreted by the kidney. Such appears not to be the case, however, since total loss of kidney function, e.g., bilateral extirpation does not produce such intoxication.

TABLE I

*Blood findings after nephrectomy*

| DOG | DATE | $CO_2$ CONTENT | $CO_2$ CAPACITY | pH | $\Sigma$ | P' | UREA | SUGAR | Cl | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *vol. per cent* | *vol. per cent* | | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | |
| I | December 2 | 43.2 | 50.6 | 7.39 | 2.3 | 6.0 | 16 | 100 | | Normal |
| | December 6 | 40.4 | 46.7 | 7.34 | 6.9 | 8.5 | 55 | 100 | | 18 hours post-operative |
| | December 8 | 40.3 | 47.7 | 7.32 | 28.2 | 10.0 | 84 | 103 | | 66 hours post-operative |
| II | December 3 | 38.9 | 45.5 | 7.41 | 4.6 | 5.0 | 16 | 120 | | Normal |
| | December 13 | 31.3 | 39.4 | 7.33 | 19.7 | 10.0 | 90 | 145 | | 70 hours post-operative |
| III | December 13 | 37.1 | 44.8 | 7.43 | 5.3 | 4.5 | 12 | 97 | | Normal |
| | December 18 | 32.3 | 43.7 | 7.36 | 30.6 | 12.5 | 83 | 110 | | 72 hours post-operative |
| IV | December 13 | 40.3 | 47.6 | 7.37 | 5.1 | 5.5 | 20 | 117 | | Normal |
| | December 18 | 33.4 | 45.1 | 7.33 | 25.7 | 10.0 | 91 | 125 | | 72 hours post-operative |
| | *1928* | | | | | | | | | |
| V | January 3 | 39.9 | 48.1 | 7.40 | 1.8 | 4.5 | 15 | 87 | 325 | Normal |
| | January 6 | 43.9 | 47.5 | 7.38 | | | | | | 72 hours post-operative |
| | January 8 | 36.5 | 40.3 | 7.35 | 31.2 | 15.0 | 100 | 105 | 220 | 105 hours post-operative |
| VI | January 9 | 41.6 | 49.0 | 7.38 | 2.7 | 5.0 | 15 | 91 | 320 | Normal |
| | January 14 | 42.8 | 48.5 | 7.38 | 30.7 | 12.5 | 85 | 133 | 240 | 72 hours post-operative |
| VII | January 9 | 40.3 | 47.8 | 7.37 | 2.5 | 5.5 | 17 | 105 | 300 | Normal |
| | January 17 | 35.5 | 42.8 | 7.38 | 23.6 | 13.2 | 100 | 118 | 240 | 98 hours post-operative |
| | January 18 | 34.6 | 41.0 | 7.38 | 37.7 | 16.2 | 112 | 122 | 221 | 120 hours post-operative |
| VIII | March 30 | 40.7 | 50.6 | 7.37 | 2.9 | 5.0 | 14 | 87 | 350 | Normal |
| | March 31 | | | | | | | | | |
| | April 1 | 39.2 | 47.6 | 7.35 | 27.7 | 12.0 | 95 | 87 | 280 | 72 hours post-operative |
| | April 2 | 32.1 | 42.5 | 7.35 | | | | | | 88 hours post-operative |

A second point of interest as shown by the data in table 1 is the fall in blood chloride which occurs following total nephrectomy. Chloride was not determined in our earlier cases. But when it became evident that acid intoxication was not present and that the animals vomited considerably, chlorides were determined.

In dog 5 the normal chloride concentration of the blood was 325 mgm. per 100 cc. Following bilateral nephrectomy (105 hours) when the animal presented marked symptoms the chlorides had fallen to 220 mgm. per 100 cc. Without exception blood chlorides diminished in the nephrectomized individuals.

Atchley and Benedict (1927) observed diminution in the chlorides following bilateral ureter ligation of dogs. They studied the chlorides lost in the vomitus and excreted in the urine and feces, but were unable to account for the loss on the basis of vomiting or excessive excretion. Study of chloride concentration of various tissues such as muscle and brain likewise failed to show deviations from the normal. They were unable to account for the fall in blood chlorides on the basis of their examinations.

Blood sulphate, phosphate, and urea increased markedly in the operated animals. Some of the values for sulphate were very high. Atchley and Benedict (1927) observed in their animals, that as the blood sulphate and phosphate rose, chlorides fell, and they state that the fall in chlorides offset the rise in sulphate and phosphate in so far as the effect upon the acid-base equilibrium was concerned.

In the present experiments one dog which survived bilateral nephrectomy 88 hours did not vomit during this interval—nevertheless the blood chlorides fell from the normal of 350 mgm. per 100 cc. to 280 mgm. seventy hours after the second operation. It seems probable, therefore, that the diminution in blood chloride can not be due to loss of chloride in vomitus.

Increase in blood sugar was observed after nephrectomy in all cases except animal 8. Animal 1 showed practically no change from normal. The remaining seven dogs showed slight increases up to the time of death. The increase in sugar was not high but sufficient to be readily detected. It appears, therefore, that a slight hyperglycemia is characteristic of nephrectomized dogs despite the fact that such animals take little or no food after symptoms develop.

Summing up the chief blood findings in bilaterally nephrectomized dogs it may be stated: 1. An uncompensated acid intoxication is not a symptom of renal insufficiency induced by kidney ablation. 2. Marked retention of sulphate, phosphate and urea occurs. 3. Blood sugar increases somewhat and remains above normal in the terminal stages. 4. Blood chlorides diminish, the decrease probably not being due to chloride lost in the vomitus since one of the dogs did not vomit but chlorides fell nevertheless.

BILATERAL ADRENALECTOMY. Detailed reports of chemical studies of

the blood of normal, unilaterally and bilaterally adrenalectomized dogs have been made elsewhere by the writer and his co-workers, and will not be repeated here except in so far as the results have a direct bearing upon the problem in hand.

It was observed that the chemical analysis of the blood of bilaterally adrenalectomized cats and dogs without symptoms showed no deviations from normal. However, when the first symptoms, such as anorexia and lassitude present themselves, a decrease in blood sugar is also present. In contrast to nephrectomized dogs whose blood sugar rises after the onset of symptoms, adrenalectomized dogs show marked lowering of the sugar of the blood when adrenal insufficiency symptoms are present. The sugar values may fluctuate up and down if considerable quantities of food such as milk are administered by stomach tube, but as the symptoms progress so also does the hypoglycemia. As the animal nears the terminal stage of adrenal insufficiency, i.e., coma, blood sugar values as low as 50 to 60 mgm. per 100 cc. are common. The hypoglycemia though marked, especially in the terminal stages, is not the cause of death, since injections of glucose although they raise the blood sugar to normal do not greatly prolong life.

Adrenal insufficiency symptoms in dogs and cats are invariably associated with acid intoxication; in fact, it is the writer's opinion that the symptoms of adrenal insufficiency are simply those of a slowly developing acid intoxication due to retention of non-volatile acids such as phosphate, sulphate and probably organic acid. Bilaterally adrenalectomized dogs may remain perfectly normal for three, four or six days following the second operation. They eat and play about in normal fashion. Chemical examination of the blood reveals nothing unusual. With the first signs of adrenal insufficiency such as total anorexia and lassitude, a marked decrease in blood sugar occurs and the $CO_2$ capacity falls without change in pH. This condition may last for a day or even two days, and as the symptoms progress the $CO_2$ capacity, $CO_2$ content and pH fall markedly. Near the terminal stages the $CO_2$ capacity may be as low as 18 vols. per cent, the $CO_2$ content 10 or 12 vols. per cent and the pH 7.10. The progress in symptoms appears to run parallel with the development of the acid intoxication.

Table 2 presents the blood findings in two typical cases. The data in this table should be compared with those in table 1 which shows the blood findings after nephrectomy. About fifty adrenalectomized dogs have been studied in this laboratory during the past year and the data given in table 2 are representative of the entire group.

In this laboratory chemical analysis of the blood is not made on animals which survive bilateral adrenal extirpation less than four days. Some of our dogs have survived 12, 10, 9, 8, 7 and 6 days. Such animals invariably

remain normal for several days following removal of the remaining gland. Many of our animals survive shorter intervals, 48, 60, 72, 80 and 91 hours. All such cases are carefully observed but detailed blood studies not made, since the survival period was considered too brief.

It is exceedingly difficult to make repeated blood determinations on adrenalectomized dogs when such large quantities of blood are necessary as in the present study, for repeated bleeding weakens the animal and

TABLE 2

*Blood findings after adrenalectomy*

| DOG | DATE OF BILATERAL OPERATION | $CO_2$ CONTENT | $CO_2$ CAPACITY | pH | N | P | SUGAR | Cl* | REMARKS |
|---|---|---|---|---|---|---|---|---|---|
| | *1927* | *vol. per cent* | *vol. per cent* | | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | |
| 38 | June 8 | 41.3 | 49.2 | 7.39 | 2.2 | 4.8 | 102 | | Normal. Bilateral 24 hours. No symptoms |
| | June 12 | 32.1 | 37.3 | 7.34 | 5.4 | 7.1 | 70 | | Anorexia, lassitude, first symptoms |
| | June 14 | 24.3 | 29.1 | 7.28 | 8.3 | 8.2 | 72 | | Acidosis. Weakness. Marked symptoms |
| | June 15 | 18.4 | 23.4 | 7.21 | 10.4 | 10.7 | 62 | | Acidosis. Marked symptoms. Survived 7 days |
| 44 | October 7 | 40.6 | 48.2 | 7.40 | 1.9 | 5.1 | 107 | | Normal. Bilateral. No symptoms |
| | October 12 | 35.2 | 40.1 | 7.39 | 2.4 | 6.1 | 103 | | Some anorexia and lassitude |
| | October 14 | 29.1 | 34.2 | 7.33 | 5.7 | 7.6 | 74 | | Symptoms. Total anorexia and lassitude |
| | October 15 | 17.4 | 21.5 | 7.17 | 11.4 | 10.1 | 65 | | Marked symptoms. Acidosis. Weakness. Survival 8 days |

* Chlorides not determined.

induces exacerbation of symptoms when they are present. It is rarely possible to obtain several sulphate and phosphate determinations on the same animal along with the other blood constituents. In only two of our animals has it been possible to do this. The sulphate determinations were usually omitted in the dogs when the other blood constituents were determined.

The data presented in table 2 show conclusively that a marked uncom-

pensated acid intoxication is characteristic of adrenal insufficiency in dogs and is apparently due to retention of phosphate and sulphate.

Chlorides were not determined in our dogs since the writer in earlier work (1927) on cats found that they do not change appreciably following adrenal removal. Lucas (1926) however, reports that in bilaterally adrenalectomized dogs, chlorides are decidedly diminished.

Urea and non protein nitrogen were not determined since it is well known that following adrenal removal in both cats and dogs these substances greatly increase (Marshall and Davis, 1917; Lucas, 1926; Swingle, 1927).

Discussion. In previous papers the writer has stated that the probable cause of death in adrenal insufficiency is a slowly advancing acid intoxication due to retention of the acid end products of normal metabolism. It was postulated on the basis of the blood analysis that the kidney fails to properly perform its acid eliminating functions in the absence of the hormone of the suprarenal cortex, and that the latter supplies something which is necessary for normal renal activity. Histological examination of the kidneys of adrenalectomized cats failed to reveal any gross pathological changes, but recently Hartmann (1927) and collaborators report lipoid nephrosis following adrenal extirpation in cats.

However this may be, it is evident from the present study of the comparative effects of total kidney removal and bilateral adrenal extirpation that the acid intoxication which is so prominent a feature of the adrenal insufficiency syndrome is not entirely dependent upon impaired kidney function since it does not appear in dogs deprived of both kidneys where failure of renal function is complete.

Phosphate and sulphate retention are even more marked in nephrectomized than in adrenalectomized animals, yet an approximately normal acid-base equilibrium is maintained.

The conditions reported for human uremics are similar to those observed following adrenalectomy in regard to the nature of the acid intoxication. In uremia, it is generally assumed that the acidosis is due to retention of phosphate and sulphate by a diseased kidney.

It seems to the writer that too much stress has been laid upon the kidney as the site of lesion responsible for the acid intoxication of both adrenal insufficiency and uremia. It is suggested here, that possibly one reason why bilaterally nephrectomized dogs maintain an approximately normal acid-base equilibrium up to the time of death, is that an extra-renal factor is responsible for the maintenance of the normal equilibrium and that the mechanism may consist of an active principle or hormone elaborated by the suprarenal cortex. In nephrectomized dogs the kidney function is eliminated but the suprarenal cortex remains intact and assists in maintaining normal equilibrium. In uremia, on the other hand, this extra-renal factor, i.e., suprarenal cortex, breaks down along with the kidney

function. In adrenalectomy the conditions would be essentially similar to those of uremia since loss of the suprarenal cortex would lead to acid intoxication, the kidney lesions being of secondary importance.

## SUMMARY

1. Bilaterally nephrectomized dogs do not develop acid intoxication. In some cases a slight fall in alkali reserve may occur but the change is in striking contrast to the acidosis which follows adrenal removal.

2. Blood sugar increases slightly after total kidney removal but falls markedly after adrenal extirpation.

3. High figures for inorganic phosphate, sulfate and urea are found after nephrectomy and adrenalectomy.

4. Blood chlorides fall as sulfate and phosphate rise following nephrectomy.

5. The suggestion is made that the acid intoxication of adrenal insufficiency and probably also of uremia is extra renal in origin since it is not present in nephrectomized dogs with total suppression of kidney function.

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# FACTORS WHICH DETERMINE RENAL WEIGHT[1]

## V. The Protein Intake

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Received for publication May 7, 1928

The observation that the ingestion of a diet containing more than the usual amount of protein is followed by an increase in the weight of the kidneys was first made by Watson and Lyon (1906) during the course of a study of the growth of the rat on a meat diet. Osborne and Mendel (1924) and many others (Drummond and Reader, 1925; MacKay, MacKay and Addis, 1925; Miller, 1925; Anderson, 1926; Jackson and Riggs, 1926) have confirmed this finding and it is now generally accepted that an increase in the protein content of the diet is uniformly followed by an hypertrophy of the kidneys. All of the investigations so far reported have been of a qualitative nature and information of a quantitative nature as to the relation of the protein intake to the size of the kidneys is lacking. An attempt has been made in the experiments reported here to obtain such quantitative information.

Methods. The general methods by which these experiments were carried out have already been described in detail (MacKay and MacKay, 1927a). Cognizance was taken of the effect of age and sex (MacKay and MacKay, 1927b, 1927c) on renal weight, factors which are often overlooked in determining experimental conditions for renal studies.

Male rats were used as the experimental animal. In each case they were removed from the mother and placed upon the experimental diet when exactly 26 days of age. Forty-four days later, that is, when the rats were 70 days old, they were etherized and killed. Anatomical measurements were then made in the manner which has been described. Although change in renal weight was the main interest the weights of the heart and of the liver were also followed in relation to the protein intake.

Three experiments were carried out in each of which the diet contained a different protein concentration. The protein concentrations were varied by the amount of casein in the diet and were roughly as 1:2:4. The mineral content and the concentrations of vitamin-containing foods were identical in each case. The diet containing the lowest protein con-

[1] This work was aided by a grant from the Ella Sachs Plotz Fund.

tent has already been described in the first paper (experimental male food) of this series. The ingredients of the two new diets are given in table 1 and the composition of all three summarized for comparison in table 2.

TABLE 1

| INGREDIENT | TOTAL | PROTEIN | FAT | CARBOHYDRATE | WATER | ROUGHAGE | SALTS | CALORIES |
|---|---|---|---|---|---|---|---|---|
| **Optimal protein diet** | | | | | | | | |
| Cornstarch | 29.0 | 0.0 | 0.0 | 26.7 | 2.3 | 0.0 | 0.0 | 109.4 |
| Casein | 32.0 | 26.6 | 0.4 | 1.4 | 2.3 | 0.0 | 0.4 | 118.6 |
| Lard | 15.0 | 0.0 | 15.0 | 0.0 | 0.0 | 0.0 | 0.0 | 139.5 |
| Cod-liver oil | 9.0 | 0.0 | 9.0 | 0.0 | 0.0 | 0.0 | 0.0 | 83.7 |
| Salt mixture | 4.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.0 | 0.0 |
| Dried yeast | 9.0 | 4.7 | 0.0 | 0.0 | 0.4 | 2.9 | 1.0 | 19.3 |
| Agar-agar | 2.0 | 0.0 | 0.0 | 0.0 | 0.2 | 1.8 | 0.0 | 0.0 |
| Total | 100.0 | 31.3 | 24.4 | 28.1 | 5.7 | 4.7 | 5.4 | 470.5 |
| Calories | | 128.0 | 227.0 | 115.0 | 0 | 0 | 0 | 470.5 |
| **High protein diet** | | | | | | | | |
| Casein | 75.0 | 62.3 | 0.9 | 3.2 | 5.6 | 0.0 | 2.0 | 276.7 |
| Cod-liver oil | 10.0 | 0.0 | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 | 93.0 |
| Salt mixture | 4.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.0 | 0.0 |
| Dried yeast | 9.0 | 4.7 | 0.0 | 0.0 | 0.4 | 2.9 | 1.0 | 19.3 |
| Agar-agar | 2.0 | 0.0 | 0.0 | 0.0 | 0.2 | 1.8 | 0.0 | 0.0 |
| Total | 100.0 | 67.0 | 10.9 | 3.2 | 7.2 | 4.7 | 7.0 | 389.0 |
| Calories | | 275.0 | 101.0 | 13.0 | 0 | 0 | 0 | 389.0 |

TABLE 2

| | PER CENT OF DIET | | | PER CENT OF CALORIES | | |
|---|---|---|---|---|---|---|
| | I Normal diet | II Optimal protein diet | III High protein diet | I Normal diet | II Optimal protein diet | III High protein diet |
| Protein | 18.0 | 31.3 | 67.0 | 15.7 | 27.3 | 70.7 |
| Fat | 24.2 | 24.4 | 10.9 | 47.7 | 48.2 | 26.0 |
| Carbohydrate | 42.1 | 28.1 | 3.2 | 36.6 | 24.5 | 3.3 |
| Water | 5.6 | 5.7 | 7.2 | 0.0 | 0.0 | 0.0 |
| Roughage | 4.7 | 4.7 | 4.7 | 0.0 | 0.0 | 0.0 |
| Salts | 5.4 | 5.4 | 7.0 | 0.0 | 0.0 | 0.0 |

RESULTS. In figure 1 have been charted for each of the three groups growth, in terms of the mean daily body weight, and food intake on the basis of the mean daily calorie and protein consumption in relation to the

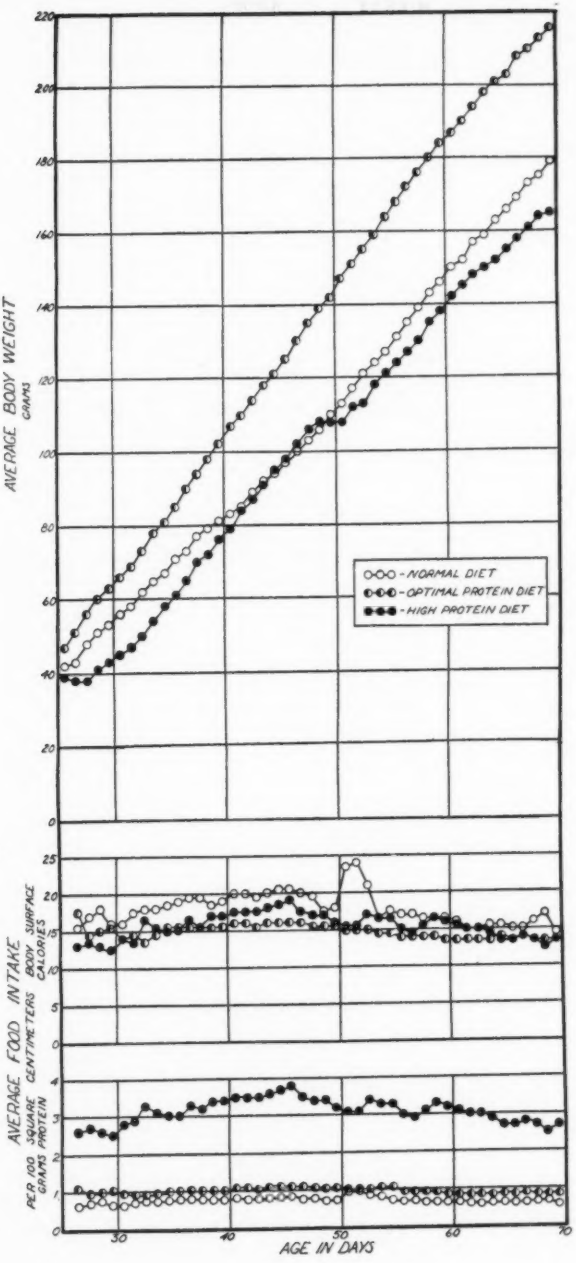
AVERAGE BODY WEIGHT
GRAMS
220
200
180
160
140
120
100
80
60
40
20
0
○○○ - NORMAL DIET
◐◐◐ - OPTIMAL PROTEIN DIET
●●● - HIGH PROTEIN DIET
AVERAGE FOOD INTAKE
BODY SURFACE
CALORIES
PER 100 SQUARE CENTIMETERS
GRAMS PROTEIN
25
20
15
10
5
0
4
3
2
1
0
30
40
50
60
70
AGE IN DAYS

Fig. 1

calculated body surface, during the period of the experiment. The group receiving the 31 per cent protein diet has by far the best growth curve

TABLE 3*

| | NUMBER OF RATS IN EXPERIMENT | BODY WEIGHT (CORRECTED) | BODY SURFACE | KIDNEY WEIGHT (AVERAGE) | HEART WEIGHT | LIVER WEIGHT | WEIGHT PER 100 CM. BODY SURFACE | | | BLOOD UREA CONCENTRATION |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Kidney | Heart | Liver | |
| **Normal diet** | | | | | | | | | | |
| | | *grams* | *sq. cm.* | *mgm.* | *mgm.* | *grams* | *mgm.* | *mgm.* | *grams* | *mgm. per 100 cc.* |
| Mean | **25** | **170** | **348** | **644** | **640** | **8.42** | **185** | **184** | **2.42** | **37.9**† |
| Standard deviation | | | | | | | 10.1 | 12.6 | 0.22 | 2.8 |
| Probable error of mean | | | | | | | 1.4 | 1.7 | 0.03 | 0.4 |
| Coefficient of variability | | | | | | | 5.5 | 6.9 | 9.21 | 7.4 |
| **Optimal protein diet** | | | | | | | | | | |
| Mean | **18** | **212** | **403** | **836** | **740** | **10.30** | **207** | **183** | **2.54** | **41.5** |
| Standard deviation | | | | | | | 18.8 | 6.0 | 0.24 | 7.4 |
| Probable error of mean | | | | | | | 3.0 | 1.0 | 0.04 | 1.2 |
| Coefficient of variability | | | | | | | 9.1 | 3.4 | 9.50 | 17.8 |
| **High protein diet** | | | | | | | | | | |
| Mean | **29** | **159** | **332** | **964** | **644** | **9.07** | **289** | **194** | **2.73** | **92.2**† |
| Standard deviation | | | | | | | 14.4 | 6.7 | 0.23 | 6.6 |
| Probable error of mean | | | | | | | 1.8 | 0.8 | 0.03 | 0.8 |
| Coefficient of variability | | | | | | | 5.0 | 3.5 | 8.16 | 7.2 |

* In order to conserve space only the average results have been given here. The authors will be glad to furnish a copy of the individual figures to any worker who may have use for them.

† Mean figure for observations from 10 rats in the group.

TABLE 4

*Group averages*

| | INTAKE—GRAMS PER 100 SQ. CM. BODY SURFACE PER DAY | | MILLIGRAMS KIDNEY PER 100 SQ. CM. BODY SURFACE | | GRAMS LIVER PER 100 SQ. CM. BODY SURFACE |
|---|---|---|---|---|---|
| | Food | Protein | Observed | Calculated | |
| Control diet group | 3.80 | 0.684 | 185 | 188 | 2.42 |
| Optimal protein group | 3.15 | 0.980 | 207 | 204 | 2.54 |
| High protein group | 3.99 | 2.670 | 289 | 295 | 2.73 |

even though their calorie intake is the lowest of the three groups. This supports the idea that the 16 per cent protein content of the original

Osborne and Mendel diet is far below the optimal figure. If the excellent growth of the group on the optimal protein diet is interpreted as being due to the higher protein content of their diet the failure of the rats on a still higher protein diet to even reach the weight curve of the controls must be explained. It is true that the high protein group consumed fewer calories than their controls but the optimal protein group consumed even less. The failure of rats to grow normally on a high protein diet has been noticed before (Drummon, Crowden and Hill, 1922) and Reader and Drummond (1926) have shown that this is due to the failure to maintain a necessary ratio between the protein and the yeast extract (vitamin B?) contents of the diet. Had the ratio of dried yeast to protein in our optimal protein

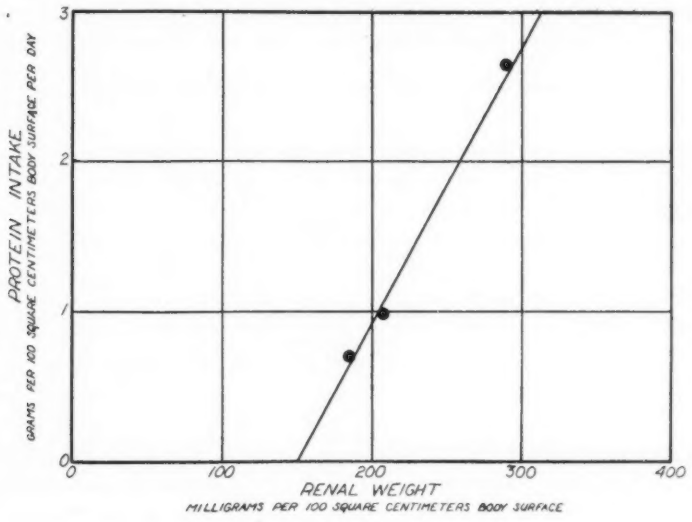


Fig. 2

diet been maintained in the high protein group we believe that their growth cu rves would have been essentially the same.

The mean results of the anatomical measurements made at death comprise table 3. From these it is very obvious that in general the larger the amount of protein in the diet the greater is the weight of the kidney but the relationship between the kidney weight and the concentration of the protein in the diets is not constant. This would hardly be expected for it is the actual protein intake which would more reasonably be the determining factor. In table 4 are the mean figures for protein consumption. These have been plotted in figure 2 against the average kidney weight of each group. This graph shows that there is a linear relationship between the

weight of the kidney and the amount of protein which is ingested per unit time which may be expressed by the formula for a straight line, $y = ax + b$, where $y$ represents the protein intake per unit of time and of body surface, $x$ the weight of renal tissue per unit of body surface, $a$ a constant dependent on the effect on the renal weight of each increment in protein intake and $b$ another constant determined by the relation of $a$ to the renal weight of a theoretical group receiving a diet devoid of protein. In the present instance $a = 0.0183$ and $b = -2.75$, that is, *protein intake = 0.0183 renal weight −2.75*. In table 4 the expected kidney weights calculated from this equation are shown to agree remarkably well with the observed figures.

Early in our work it occurred to us that the relationship found between protein intake and renal weight was a result of the demand made on the kidney in relation to nitrogen excretion. This has been shown (MacKay,

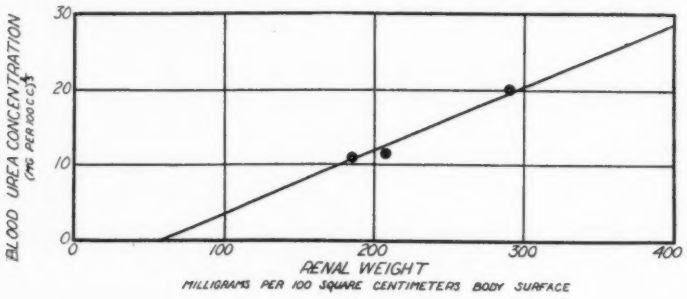


Fig. 3

MacKay and Addis, 1927) to be at most only a contributing factor. Another possibility which presented itself was that the increase in kidney weight which follows an increased protein intake may be by virtue of the increased blood urea concentration. The concentration of urea in the blood was determined in a majority of the rats when they were killed in all of the present experiments. In order to place the blood urea figures on a comparable basis with the renal weight figures as a function of body surface they have been taken to the two-thirds power and plotted against renal weight in figure 3. The relationship is seen to be a linear one. It might be assumed then that the protein intake influenced renal weight through its effect on the blood urea concentration. Since, however, it will be shown in other experiments of this series that this relationship is present only when the blood urea concentration is increased by varying the protein intake and does not hold when urea ingestion is the source of the increased blood urea concentration we must conclude that this is not a causal

relationship and exists solely because both the renal weight and the blood urea concentration bear a constant relation to the protein intake.

Numerous observations reported in the literature indicate that high protein diets increase the weight of the liver but in recent experiments with adults rats (Moise and Smith, 1927) no such increase has been observed. Although not greatly significant from a statistical point of view the average figures of the present experiments (table 4) indicate that the higher the protein intake the heavier the liver. The weight of the heart, on the other hand, appears to be uninfluenced by variations in the protein intake.

## SUMMARY

A linear relationship between renal weight and protein intake has been demonstrated. For normal male rats 70 days of age receiving adequate amounts of protein in the form of casein it may be expressed: *protein intake* (grams per 100 sq. cm. body surface per day) = *0.0183 renal weight* (milligrams per 100 sq. cm. body surface) $-2.75$.

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# FACTORS WHICH DETERMINE RENAL WEIGHT[1]

## VI. Influence of Age on the Relation of Renal Weight to the Protein Intake and the Degree of Renal Hypertrophy Produced by High Protein Diets

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Received for publication May 7, 1928

In the preceding paper the observation that an increase in the protein intake is followed by an increase in the size of the kidneys has been confirmed. It was further shown that in young animals this increase is of such degree that a constant relation between renal weight and protein intake can be established. Age has previously been shown (MacKay, MacKay and Addis, 1925) to markedly influence the degree of compensatory renal hypertrophy which follows the removal of one kidney. With this in mind the influence of age on the degree of renal hypertrophy produced by high protein diets and on the relation of renal weight to protein intake has been determined.

The methods and diets which were used have been described in other papers of this series. In the preceding paper three adequate diets which contained 18 per cent, 31 per cent and 67 per cent of protein respectively were fed to three groups of male albino rats from the time they were 26 days old until they were 70 days of age. At this time the relation of the protein intake to renal weight was determined. In the present experiment the same diets were given to adult rats from the time they were 346 days of age until they were 400 days old. At the end of this period measurements were made in a manner identical with that used on the young rats. The figures for daily body weight, food intake in terms of calories, and protein consumption in relation to body surface are charted for the three groups in figure 1. Unlike the young rats, the rate of growth of the adult rats on the optimal protein (31 per cent) diet is no greater than the rate of growth of those on the normal (18 per cent protein) diet. In relation to the other two groups the rats on the high protein diet behaved exactly as the younger rats, the weight gain for this group being less than that for the other two.

[1] This work was aided by a grant from the Ella Sachs Plotz Fund.

In table 1 are recorded the average results of anatomical data obtained when the animals were killed at 400 days of age. In figure 2 have been plotted the average kidney weights and average protein consumption figures from the data given for young rats (70 days of age) in the preceding paper and they have been fitted with the line which gives the linear relationship between the kidney weight and the protein intake. On the

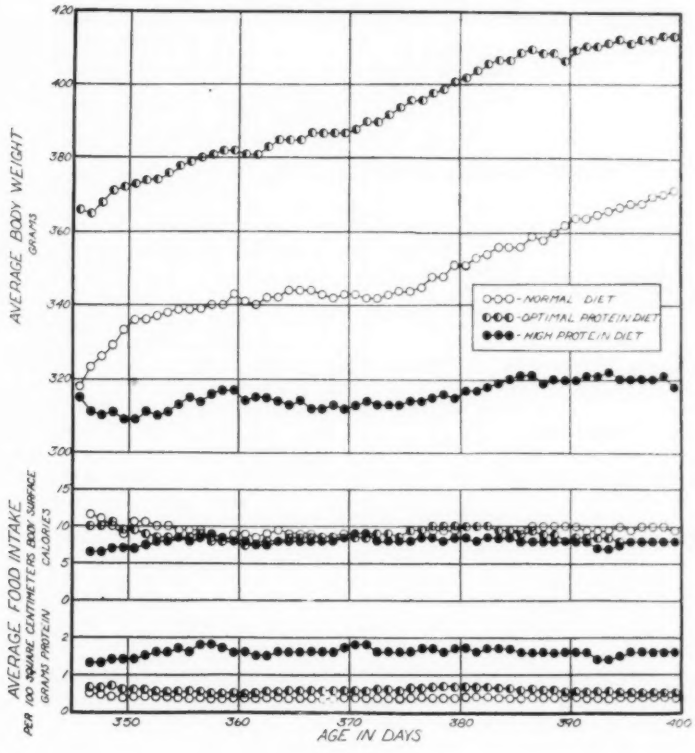


Fig. 1

same chart have been plotted the average observations of the 400-day old groups (table 2). They are found to coincide almost exactly with the relationship for the earlier age where *protein intake = 0.0183 renal weight −2.75*. The calculated renal weights determined by this formula are compared with the observed weights in table 2. The differences are very small. We may then conclude that age has no influence on the linear relationship which exists between renal weight and the protein intake.

The relation of renal weight to the blood urea concentration was noted for young rats. The graph showing this relationship is reproduced in figure 3 and similar data from the 400-day old rats have been added to it. They fit fairly well. Here again we have a relationship which exists because both the renal weight and blood urea concentration are factors whose magnitude is dependent on the protein intake.

TABLE 1*

| | NUMBER OF RATS IN EXPERIMENT | BODY WEIGHT (CORRECTED) | BODY SURFACE | KIDNEY WEIGHT (AVERAGE) | HEART WEIGHT | LIVER WEIGHT | WEIGHT PER 100 SQ. CM. BODY SURFACE | | | BLOOD UREA CONCENTRATION |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Kidney | Heart | Liver | |
| **Normal diet** | | | | | | | | | | |
| | | *gms.* | *sq. cm.* | *mgm.* | *mgm.* | *gms.* | *mgm.* | *mgm.* | *gms.* | *mgm. per 100 cc.* |
| Mean | 22 | 367 | 581 | 1,035 | 949 | 12.89 | 178 | 163 | 2.21 | 28.1† |
| Standard deviation | | | | | | | 15.8 | 15.4 | 0.19 | 3.4 |
| Probable error of mean | | | | | | | 2.3 | 2.2 | 0.04 | 0.5 |
| Coefficient of variability | | | | | | | 8.9 | 9.4 | 8.44 | 12.1 |
| **Optimal protein diet** | | | | | | | | | | |
| Mean | 13 | 410 | 627 | 1,102 | 1,014 | 13.40 | 176 | 161 | 2.14 | 35.1 |
| Standard deviation | | | | | | | 10.4 | 9.8 | 0.16 | 4.7 |
| Probable error of mean | | | | | | | 2.0 | 1.8 | 0.03 | 0.9 |
| Coefficient of variability | | | | | | | 5.9 | 6.1 | 7.62 | 13.4 |
| **High protein diet** | | | | | | | | | | |
| Mean | 26 | 312 | 521 | 1,145 | 910 | 11.36 | 219 | 175 | 2.16 | 62.2‡ |
| Standard deviation | | | | | | | 14.5 | 12.4 | 0.22 | 12.4 |
| Probable error of mean | | | | | | | 2.0 | 1.7 | 0.03 | 1.7 |
| Coefficient of variability | | | | | | | 6.6 | 7.1 | 10.20 | 20.0 |

* In order to conserve space only the average results have been given here. The authors will be glad to furnish any worker who may have use for them with a copy of the individual figures.

† Mean figure for observations from 18 rats in the group.

‡ Mean figure for observations from 24 rats in the group.

Since the relation of renal weight to the protein intake is the same in young and adult rats a given actual increase in protein intake will give the same actual increase in renal weight at both ages. On the other hand, an increase of a given percentage in the protein intake will cause a larger percentage and actual increase in the renal weight of young rats because the protein intake per unit of body surface for a given diet is much lower in adult rats while renal weight as a function of body surface is essentially

the same at all ages. The difference is well shown in figure 4 which is based on the highest and lowest protein intakes in the data comprising figure 2. In rats 70 days of age a 100 per cent increase in protein consumption leads to an increase of 19.5 per cent in renal weight while a similar increase in the protein intake of 400 day old rats is followed by only an 8.9 per cent increase in renal weight.

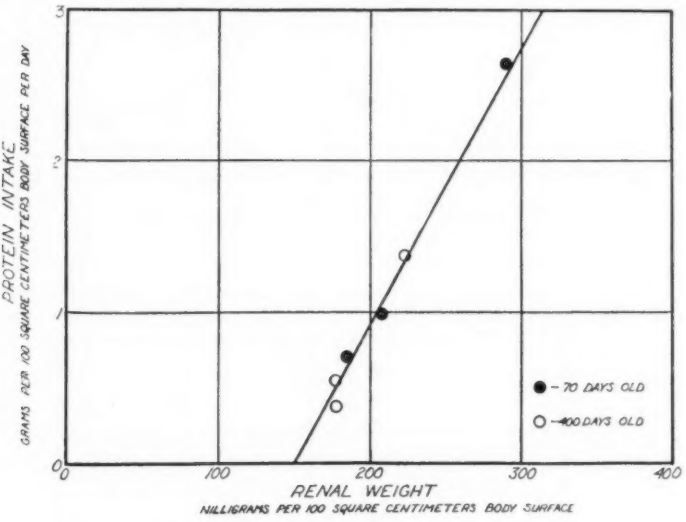


Fig. 2

TABLE 2

*Group averages*

| | INTAKE-GRAMS PER 100 SQ. CM. BODY SURFACE PER DAY | | MILLIGRAMS KIDNEY PER 100 SQ. CM. BODY SURFACE | | GRAMS LIVER PER 100 SQ. CM. BODY SURFACE |
|---|---|---|---|---|---|
| | Food | Protein | Observed | Calculated | |
| Control diet group | 2.01 | 0.362 | 178 | 170 | 2.21 |
| Optimal protein group | 1.79 | 0.560 | 176 | 181 | 2.14 |
| High protein group | 2.05 | 1.374 | 219 | 224 | 2.16 |

In young rats it was found that the higher the protein intake the heavier the liver. It was noted that Moise and Smith (1927) concluded that the liver weight was not affected by an increased protein intake. These investigators used adult rats and in the present experiment where the groups are composed of rats 400 days of age the liver weight was

found to be unmodified by large increases in the protein intake. As with the rats 70 days old the heart weight of the adult group receiving the highest protein intake is slightly higher than that of either of the groups on the other two diets, although the increase in either case is hardly significant.

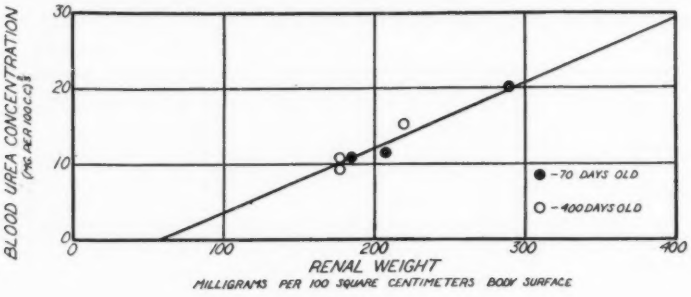


Fig. 3

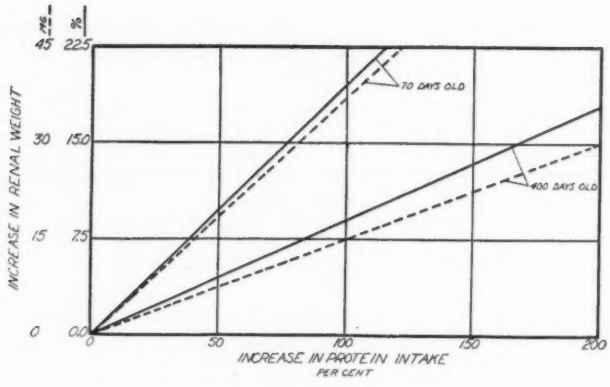


Fig. 4

## SUMMARY

Age has no influence on the linear relationship between renal weight and the protein intake. The linear formula, *protein intake = 0.0183 renal weight −2.75*, is a satisfactory expression of the relationship not only for young male rats 70 days of age but also for adult rats 400 days old when the protein intake consists of casein in adequate amounts.

The percentage and actual increase in renal weight which follows a given percentage increase in protein intake decreases with increased age.

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# THE ACID INTOXICATION OF ADRENAL INSUFFICIENCY IN DOGS

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Received for publication June 5, 1928

Considerable work has been done in recent years on the functional significance of the suprarenals, but not until comparatively recently have any results been forthcoming which would justify the expenditure of time and energy involved. Numerous investigators turned their attention to the cortical portion of the suprarenal following the work of Biedl (1903) which clearly demonstrated the incompatibility of removal of all cortical tissue with life. This work has been amply confirmed by later investigators (Wheeler and Vincent, 1917; Houssay and Lewis, 1923; Wislocki and Crowe, 1924; Zwemer, 1927; Swingle and Eisenmann, 1927), all of whom demonstrated that complete removal of adrenal cortical tissue results in early death.

The investigation of Swingle and Eisenmann was carried out on cats, but owing to the fact that cats are very difficult to bleed and, furthermore, that large quantities of blood are necessary for repeated analysis, their results, as the senior author states, are not as complete as might be desired. A review of their tables and protocols reveals that an uncompensated, non-volatile acidosis exists in adrenalectomized cats. It was with the idea of studying the nature of this acidosis following suprarenalectomy, and especially the beginning and intermediate stages, that this investigation was undertaken.

Because of the large quantities of blood necessary for repeated analyses and because of the relative ease with which a larger animal can be bled, the dog was chosen as a subject for this investigation.

The writer takes this opportunity to express his gratitude to Dr. W. W. Swingle not only for suggestion of the problem but also for his encouragement and whole-hearted enthusiasm during the progress of the work.

MATERIAL AND METHODS. Only adult and non-pregnant dogs were employed since pregnancy is known to greatly prolong the survival period of some dogs following ablation of cortical tissue (Stewart and Rogoff, 1927). Whereas the normal survival period ranges from $5\frac{1}{2}$ to 6 days, pregnant dogs are on record with survivals into the 57th and 58th days.

TABLE 1

*Blood findings following bilateral suprarenalectomy in dogs*

| CASE | DATE | TIME | $CO_2$ CAPACITY | pH | BLOOD SUGAR | INORGANIC PHOSPHORUS | REMARKS |
|---|---|---|---|---|---|---|---|
| | | | *vol. per cent* | | *mgm.* | *mgm. per 100 cc.* | |
| D19 | January 25 | 10:00 p.m. | | | | | Right gland removed |
| Mongrel | February 4 | 10:00 p.m. | | | | | Left gland removed |
| Male | February 5 | 10:00 p.m. | 49 | 7.34 | 105 | 5 | Normal blood, good recovery |
| | February 9 | 11:30 p.m. | | | 85 | | Vomiting, moody, not weak |
| | February 10 | 9:30 a.m. | 35 | 7.35 | 62 | 10 | Very weak, hind legs spreading. Respiration deep and full |
| | February 10 | 3:00 p.m. | 23 | 7.17 | 62 | 10 | Prostrate. Respiration 32 per minute and very deep. Died at 3:30 p.m. Survived 137 hours |
| D21 | February 4 | | | | | | Right gland removed |
| Female | February 11 | 8:30 p.m. | | | | | Left gland removed |
| Terrier | February 13 | 10:00 a.m. | 44.4 | 7.36 | 95 | 4.25 | Normal, eating well |
| | February 15 | 10:00 a.m. | 41.4 | 7.37 | 77 | | Venous.* Refused food, moody, vomiting |
| | February 15 | 2:00 p.m. | 33 | 7.32 | | | Respiration deeper, 24 per minute |
| | February 15 | 9:30 p.m. | 28.6 | 7.27 | 83 | 6.75 | Respiration deep with sighs |
| | February 16 | 5:00 a.m. | 24.4 | 7.19 | 62 | 13 | Prostrate. Died at 8 a.m. Survived 108 hours |
| D23 | February 12 | | | | | | Right gland removed |
| Female | February 17 | 2:00 p.m. | | | | | Left gland removed |
| Collie | February 19 | 10:00 p.m. | 46 | 7.43 | 109 | 5.3 | Normal, taking meat and milk |
| | February 22 | 10:00 a.m. | 38 | 7.42 | 67 | | Refused food, moody and weak |
| | February 22 | 10:00 p.m. | | | 78 | | Respiration 20 per minute, vomiting |
| | February 22 | 12:00 p.m. | 37 | 7.40 | | | Weaker. Respiration 24 per minute. Deeper |
| | February 23 | 3:30 a.m. | 29 | 7.17 | 64 | 10 | Restless, "flat." Died 4 a.m. Survived 134 hours |
| D24 | February 19 | | | | | | Right suprarenal removed |
| | | | | | | | Left suprarenal removed |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D24 | February 19 | | | | | | Right suprarenal removed |
| Brown | February 25 | 11:30 a.m. | | | | | Left suprarenal removed |
| Bull | February 27 | 2:00 p.m. | 40.5 | 7.38 | 100 | 4.5 | Normal, eating salmon, drinking milk |
| | February 28 | 3:00 p.m. | 32.3 | 7.36 | 76.9 | | Quiet, moody, walks little, no food, respiration shallow |
| | February 28 | 11:45 p.m. | 31.4 | 7.30 | 86.9 | | Tired and cuddled up. Respiration deep and full, 20 per minute |
| | March 1 | 10:30 a.m. | | | 62.5 | | Breathing deeper, 24 per minute. Milk forced |
| | March 1 | 2:30 p.m. | 27.6 | 7.27 | 80 | | Respiration 40 per minute. Lying flat at 3 p.m. |
| | March 1 | 4:00 p.m. | 19 | 7.24 | 55 | 6.3 | Involuntary bowel movement. Died at 4:30 p.m. Survived 101 hours |
| D26 | March 2 | | | | | | Right suprarenal removed |
| Female | March 12 | | | | | | Left suprarenal removed |
| Spitz | March 14 | 9:30 p.m. | 40.3 | 7.38 | 111.1 | 4.72 | Normal, eating and drinking |
| | March 15 | 9:30 p.m. | | | 100 | | Muscle twitchings |
| | March 16 | 4:00 a.m. | 37.4 | 7.32 | 80 | | No food, hind legs weak |
| | March 16 | 9:00 a.m. | | | 93 | | Cerebellar symptoms swaying |
| | March 16 | 2:15 p.m. | 37 | 7.34 | 94 | | Eating meat again. Twitching of hind limb muscles |
| | March 17 | 10:00 a.m. | 35 | 7.33 | 108 | | Eating, yelling fits. Respiration shallow |
| | March 18 | 9:30 a.m. | 27 | 7.27 | 76.8 | | No food, legs very weak. Respiration deep, 14 per minute |
| | March 18 | 7:15 p.m. | 22 | 7.10 | 66 | 11.12 | Venous.* Respiration deep and full. Survived 148 hours |
| D27 | March 18 | | | | | | Right gland removed |
| Male | March 26 | 3:00 p.m. | | | | | Left gland removed |
| Spitz | March 28 | 8:00 p.m. | 46 | 7.36 | 105 | 4.3 | Eating meat, running around |
| | March 30 | 9:00 p.m. | 39 | 7.31 | 83 | | No appetite, tired and moody |
| | March 31 | 9:15 a.m. | 29 | 7.28 | 87 | 5.6 | Muscle twitchings, respiration 16 per minute, deep and full, 50 cc. blood drawn |
| | March 31 | 11:00 a.m. | 27 | 7.10 | 64 | 7.7 | Respiration very deep and full, 22 per minute. Death hastened by excessive bleeding at 9 and 11 a.m. Survived 116 hours |

* Venous blood is two (2) volumes per cent higher than arterial blood.

TABLE 1—*Concluded*

| CASE | DATE | TIME | $CO_2$ CAPACITY | pH | BLOOD SUGAR | INORGANIC PHOSPHORUS | REMARKS |
|---|---|---|---|---|---|---|---|
| | | | *vol. per cent* | | *mgm.* | *mgm. per 100 cc.* | |
| D28 | January 2 | | | | | | Left suprarenal removed |
| Male | January 10 | Noon | | | | | Right suprarenal removed |
| Mongrel | January 13 | 8:00 p.m. | | | 117 | 3.8 | Fine shape; blood for $CO_2$ and pH lost in centrifuge |
| | January 15 | 2:00 p.m. | 51 | 7.39 | | | Very good condition, very active |
| | January 18 | 7:30 p.m. | 34 | 7.35 | 76 | | At 4 p.m. weak and staggering. No food-glucose injected |
| | January 19 | 12:45 a.m. | 29 | 7.19 | 91 | 12.5 | Respiration very deep and full. Distressed and "flat" |
| | January 19 | 1:30 a.m. | | | | | Too much blood loss. Died in convulsions. Survived 205 hours |
| D30 | March 2 | | | | | | Left gland removed |
| Male | March 9 | 9:00 a.m. | | | | | Right gland removed |
| Mongrel | March 13 | 8:00 p.m. | | | | | Eyes bloodshot but no weakness or food refusal |
| | March 14 | 3:00 p.m. | 33.6 | 7.30 | 87 | 9.7 | Weak and moody but took little milk after bleeding. Fed by stomach tube. Walks well but not far. Inclined to lie around |
| | March 15–16 | | | | | | |
| | March 17 | 9:00 a.m. | 18.4 | 7.22 | 60 | 16.2 | Prostrate and approaching coma. Died after bleeding. Survival—192 hours |
| D37 | April 9 | | | | | | Right suprarenal removed |
| Male | April 14 | 3:00 p.m. | | | | | Left suprarenal removed |
| Mongrel | April 16 | 10:00 a.m. | 45 | 7.41 | 91 | 3.1 | Normally acting dog |
| | April 18 | 2:30 p.m. | 34 | 7.42 | 78 | 5 | No food, respiration deep. Dog moody and lying about |
| | April 18 | 8:00 p.m. | | | 77 | 6.25 | Vomiting. Respiration 16 per minute |
| | April 19 | 10:30 a.m. | 32 | | 77 | | Very deep respiration. Very moody |
| | April 20 | 4:00 p.m. | 28 | 7.16 | 62 | 7.5 | Prostrate. Died at 5:30 p.m. Survived 146.5 hours |

In this work one pregnant dog was encountered which lived, however, only 9 days. At autopsy this dog showed 4 very young embryos. Another bitch after 27 days' survival was sacrificed and very careful autopsy revealed only one suspicious looking nodule which upon sectioning, however, had no semblance of adrenal tissue. Through accident the dog was removed from the animal room before early pregnancy could be ascertained so this animal remains unclassified. It is obvious that pregnant dogs could not have been employed in this series of experiments without seriously complicating the interpretation of results.

The removal of the second adrenal followed the first operation after a lapse of 5 to 10 days, or, as in some cases, after a longer interval. Longer intervals between operations apparently do not prolong the survival period of dogs. Ordinarily no great difficulty was experienced with gland extirpation, although occasional animals presented some difficulty in removal of the right gland. The same method of approach and removal was employed as reported by Banting and Gairns (1926).

All blood was drawn directly from the heart without allowing the animal to struggle. Blood drawn during struggling was discarded since muscular activity is known to alter the level of certain blood constituents including lactic acid, $CO_2$ and blood sugar. Blood for $CO_2$ capacity and pH was drawn under oil and determination of these factors was made before inorganic phosphorus and blood sugar were estimated. Normal blood was at first drawn from unilaterally operated animals since this blood presents no change from normal (Swingle, 1927; Lucas, 1926). Later, however, normal blood was obtained after the second operation. Doubly operated dogs of average survival are always normal for several days following removal of the second gland. They eat and occasionally fight with one another just as do unoperated or unilaterally operated animals. The blood pictures of these animals show no deviations from the normal blood picture of unoperated or unilaterally operated dogs (Yonkman, 1927), supporting the contention of other investigators (Rogoff and Stewart) that doubly operated dogs show complete operative recovery in that they are normally acting animals. Following the onset of characteristic symptoms of adrenal insufficiency as described below and elsewhere (Rogoff and Stewart, 1926; Zwemer, 1927), blood was taken at various intervals such as time of mild symptoms, more severe symptoms and terminal stages of adrenal insufficiency.

The $CO_2$ capacity of the plasma was determined by Van Slyke's (1917) method and the pH by means of Myer's bicolorimeter (1922) using Muntwyler's (1927) correction of 0.30 for dog plasma. Inorganic phosphorus of the serum was determined by the Benedict-Theiss (1924) method and blood sugar according to the method of Folin and Wu (1919).

Symptoms. The symptoms of suprarenal insufficiency are readily

observed in the dog, the first to appear being those of anorexia and weakness. Weakness is evident from the slight swaying that may be seen as the dog walks about. Often the animal walks but little and soon drops into a huddle in its cage. The animal is not as active as when no symptoms are present and often the weakness may be accompanied by refusal of food. Occasionally at this stage the dog attempts to chew or suck at a rarebit but the jaws are too weak to complete the process.

Following the onset of symptoms the dog becomes restless, carries a worried expression, is hypersensitive and often objects to being disturbed. When made to stand, the legs tend to spread and the head droops. The eyes become bloodshot, and pupils dilate and the animal presents a condition that can be no better described than by the expression—"hang-dog." Very often the animal upon being disturbed runs wobbling and swaying over to a corner of the room or back of a cage and there presents the typical "hang-dog" condition or slouches down in apparent distress. Heavy sighs give evidence that the effort required much exertion—the respiration often becoming faster and irregular.

Yelling fits of a peculiar type often accompany the condition of hypersensitivity. The least disturbance sends the dog on a wild excursion, bumping into cages and walls and from all appearances and from the noises elicited one would conclude that the animal was suffering from some wild hallucination. Rogoff and Stewart (1926) describe the same peculiar yelling fits and suggest the possibility of intestinal worms being at the seat of the disturbance. They further state that they believe some of the yelling fits are actually due to adrenal deficiency. Some dogs in this laboratory have been observed to suffer from yelling fits but at autopsy showed no worms in the entire intestinal tract. This condition was especially true of pups which in some cases suffered from these fits even after unilateral operation. These wild yelling fits are never observed in cats and were observed only in some of the dogs included here. Thus it would seem that some factor, other than the disturbance of the gut tract, may be the cause of this nervous phenomenon. What this factor may be is only a matter of conjecture unless it be suprarenal insufficiency.

The refusal of food may often be accompanied by vomiting of varying degree. In some cases vomiting may be delayed until marked weakness prevails. The vomitus is usually yellow and viscid and is often frothy and may contain blood. Not infrequently at time of vomiting bloody stools may be passed, the amount of blood increasing with severity of symptoms.

Forcing of fluid may be beneficial in some cases after food has been refused provided the forcing is not too long delayed. Once vomiting occurs early death is probable and any attempts at temporary relief by feeding milk and glucose or other liquid nutriment are usually futile.

Early symptoms may appear 24 to 36 hours before death but at times the decline is very abrupt. D22 is an animal which first showed symptoms at 10 p.m. of the 4th day of the survival period. It was judged from the blood picture that the dog would survive the night but at 8 a.m. the following morning the animal was found dead in its cage. The body was still warm and death was placed at 7 a.m. This is the shortest survival period following initial symptoms that has been observed by the writer and shows how rapidly an animal suffering from suprarenal insufficiency can pass through the stage of deficiency.

After vomiting occurs an exacerbation of symptoms follows rather quickly, including noticeable changes in depth and rate of respirations. Muscular twitchings, especially in the hind legs, are often observed, the severity of the tremors increasing with the severity of the other symptoms. Prostration is of short duration, the animal being completely exhausted and after only a few hours passing into coma and dying in convulsions which are usually accompanied by involuntary bowel movement with passage of bloody contents. Little urine is passed during the period of insufficiency but when passed it is very concentrated and has a characteristic, strong odor.

Some of the animals surviving but 2 and 3 days unquestionably died from the effects of surgical shock—at least shock was a contributing factor. The symptoms of these animals are somewhat different from those of dogs surviving bilateral removal of the suprarenals for several days. The shocked dog never eats following operation; it lies around in its cage and moves but little. Its eyes seldom become bloodshot to the extent that occurs following long survival, and muscular tremors, if present at all, are much less marked. Bloody stools may be present but the typical yellowish and frothy fluid of insufficiency of long survival is lacking.

At autopsy, besides the presence of the yellow fluid described above, there are other differences which distinguish between the shocked dog and the animal suffering from typical adrenal insufficiency. The spleen and pancreas show congestion but the spleen is noticeably hard and enlarged or elongated in the long survival. The liver of the latter animal is extremely soft and mushy and the gall bladder is more distended in the long survival than in the shocked animal.

The kidneys present no evident gross change following bilateral adrenalectomy.

Blood findings. *Acidosis.* The blood findings indicate that with the onset of symptoms of adrenal insufficiency such as refusal of food, muscular weakness and general lethargy, the initial fall in $CO_2$ capacity occurs, whereas the pH remains at normal level or at least within the normal range. With continued fall in $CO_2$ capacity the pH remains normal until the capacity has fallen to approximately from 30 to 33 volumes per cent.

At this level the first decline in pH is usually observed and when the level of $CO_2$ capacity falls below 30 vol. per cent a rapid decline in pH ensues. The gradual decrease in $CO_2$ capacity is accompanied by a corresponding increase in depth, and in the later stages, in the number of respirations. There comes a time, however, when respiratory compensation is inadequate and a sharp decline in pH follows, this change occurring between 4 and 12 hours preceding death.

*Blood sugar.* With the onset of symptoms the initial fall in $CO_2$ capacity is accompanied by a marked drop in blood sugar which falls to approximately 75 to 80 mgm. per cent of blood. The possibility remains that the sugar may drop before the capacity thus not necessarily accompanying the $CO_2$ capacity decline. The sugar level may fluctuate materially once it begins to fall and in two of the several cases approached the normal level (D24 and D26).

D24 is a dog which showed fluctuation of the blood sugar level before and after forceful feeding. Examination of the protocols reveals slight fluctuations in blood sugar levels but none as marked as here cited. Regardless of the fluctuations the sugar level invariably drops in the acute stages preceding coma, in some cases to as low as 55 to 60 mgm. per cent. It seems probable that the early symptoms of adrenal insufficiency such as anorexia, languor and first signs of weakness are due to the initial decline in blood sugar. Every case in the protocols reveals a low blood sugar at time of first signs of weakness and the levels of blood sugar are sufficiently low in the respective animals to account for the degree of weakness noted.

*Phosphorus.* The inorganic phosphorus of the serum shows a gradual rise with the onset of symptoms but the rise is most rapid in the later stages. An examination of D21 shows a normal phosphorus of 4.25 mgm. per cent with a slight increase to 6.75 mgm. at the time the acidosis changed from the compensated to the uncompensated condition. Seven hours later with a pH of 7.19 the phosphorus level had risen to 13 mgm. Had the phosphorus been determined again three hours later, just one hour before death, perhaps a still higher level would have been observed. Lucas (1926) cites a dog surviving 217 hours and having a phosphorus of 26.1 mgm. This is higher than any encountered in this series of observations, the highest ever observed here being 16 mgm. per cent (D30).

*Renal function tests and albumin.* Excretion in the kidney was determined in three cases by means of the Rowntree and Geraghty (1910) method. In a cat living 4 days, $4\frac{1}{2}$ hours, normal excretion amounted to 65 per cent. Three days after the second operation the output had decreased to 28 per cent, the animal showing slight signs of weakness. On the fourth day with marked weakness prevailing excretion had further diminished to 12 per cent.

In a dog, kidney output fell from normal 84 per cent to 60 per cent at time of first symptoms and in another cat it fell from normal 59 per cent to 37 per cent on the third day after the second operation and the cat showing no signs of weakness whatever.

The data on renal function tests are too meager to be of much significance and are included here for what they may be worth. The single case of the 4 day cat is clean cut and shows clearly a greatly diminished output of the dye following the onset of suprarenal insufficiency symptoms. However, the significance of these figures in the absence of blood pressure determinations is doubtful. The writer intends to continue this work.

In none of the bilaterally adrenalectomized dogs was any albumin found in the urine.

Discussion of blood findings. In this investigation attention was concentrated upon the $CO_2$ capacity, pH, inorganic phosphorus and blood sugar,—the three factors first named because they concern themselves with acidosis, and the latter because it seemed desirable to account for the initial symptoms by means of the blood picture. No determinations were made for chlorides, urea, non-protein nitrogen and other blood constituents since detailed reports concerning these components are given elsewhere (Swingle, 1927; Swingle and Eisenmann, 1927; Rogoff and Stewart, 1926; Lucas, 1926; Banting and Gairns, 1926). The rôle that some of these constituents probably play is commented upon by the respective authors.

Whatever toxic agents be present may be temporarily dispelled or diluted as shown by the work of Banting and Gairns (1926) who report transient relief following injections of saline solutions. Prolongation of the survival period of suprarenalectomized cats is effected by oral administration of various fluids (Corey, 1927) and in dogs by intravenous injections of normal saline and 5 per cent glucose (Rogoff and Stewart, 1926). Apparently the basis for all of this work is the relief of the dehydration which occurs in adrenalectomized cats and dogs and perhaps the diuretic effect of the fluids administered is a factor (Banting and Gairns, 1926). Rogoff and Stewart (1926) suggest the possibility of the intestine excreting the toxic substance or substances and further state that if this is true, the pathological condition of the gut at autopsy is largely accounted for.

As the data indicate, and as Swingle discusses in his paper (1927), there remain two possibilities accounting for the death of suprarenalectomized animals, namely, the hypoglycemia and the acidosis. That hypoglycemia is but a contributing factor and not the sole cause of death has been demonstrated by relieving the hypoglycemia with glucose injections (Swingle, 1927). Cats, thus injected, die in spite of a normal blood sugar.

Banting and Gairns (1926) removed the adrenals from depancreatized dogs and observed that their animals died with nitrogen retention and high blood sugar. These data preclude the conclusion that death results from hypoglycemia although it is evident that it is a contributing factor.

It is of interest to note that weakness and lassitude which are first to appear in the train of typical adrenal insufficiency symptoms are directly associated with a fall in blood sugar. The sugar level at this stage falls as low as 75 mgm. per cent. In most of the cases recorded there is a gradual decline in blood sugar after the initial fall occurs but D24 and D26 present evidence that the sugar level may fluctuate, apparently depending upon whether or not food is taken voluntarily or is forced.

The point worthy of emphasis, however, and bearing repetition, is that refusal of food, weakness and general fatigue seem directly attributable to a low blood sugar, this level varying with different animals. Swingle (1927) has shown that these symptoms can be partially alleviated by subcutaneous injections of glucose and insulin but the effects are transient and soon wear off, marked symptoms being associated with a gradual lowering of the blood sugar level until death supervenes.

The other possibility—that of acidosis, is of sufficient magnitude to cause death without taking into account the accompanying hypoglycemia. although the latter, as indicated, may be a contributing factor.

It was observed that acidosis could be readily followed though not with accurate nicety, by means of the visible changes in respiration. Before much decrease in $CO_2$ capacity had occurred no appreciable variation in respiration had taken place. However, when respiration became deeper and slower the blood picture showed significant decreases in $CO_2$ capacity. Approaching the level when the compensated acidosis with low $CO_2$ capacity and normal pH shifts to the uncompensated condition with falling pH, respiration became deeper and more rapid until in the acute stages, with lowering pH and continued fall in $CO_2$ capacity, respiration was deepest and most rapid until the animal passed into coma. This change in respiration and failure of respiratory compensation served conveniently as a rough indicator of the degree of acid intoxication.

That the $CO_2$ capacity determination of the plasma is of little value unless the pH is also considered is seen from the recent work of Pico and Deulofeux (1926) who report that following suprarenalectomy in dogs "the carbonic anhydride diminished, especially in the arterial blood, because of hyperpnea." This would result in a condition of alkalosis with rising pH in contradistinction to the condition of acidosis with falling pH reported here. It is obvious that with $CO_2$ content and $CO_2$ capacity determinations the pH of the blood must be considered and that no reliance can be placed on $CO_2$ readings alone.

In a preliminary report (1927) it was suggested that the acidosis was

due to retention of the acid end-products of normal metabolism. Failure of the kidney to excrete acid phosphate and organic acids (Swingle, 1927) partially accounts for the acidosis following bilateral adrenalectomy in cats. In dogs the gradual rise in inorganic phosphorus of the serum plays an active rôle in the condition known as acidosis,—its major action being exerted in the uncompensated condition when the rise is most rapid. However, this increase in inorganic phosphorus is not sufficient to account for the degree of acidosis present and further light is shed on the factors contributing to the acidosis by the recent work of Swingle and Wenner (1928) who observed a marked increase in inorganic sulphate in adrenalectomized dogs. The phosphate and sulphate retention when considered together probably largely account for the degree of acidosis present. That HCl is hardly a factor to be considered as contributing to the acidosis is seen from the work of Rogoff and Stewart (1926) and Lucas (1926) who report that blood chlorides are often moderately diminished, and from Swingle (1927) who reports chlorides remain practically unchanged in cats. Future work should include determination of lactic acid and other organic acids.

The acidosis of adrenalectomy presents a marked similarity to the acidosis of chronic interstitial nephritis. In both conditions phosphate and sulphate retention contribute to the uncompensated acidosis observed in the terminal stages.

The fact that the blood picture following bilateral suprarenalectomy reveals a condition of acid intoxication, retention of nitrogenous constituents, inorganic phosphorus and inorganic sulphate, indicates that severe renal impairment of some sort probably follows as a direct consequence of loss of adrenal cortical tissue.

## SUMMARY AND CONCLUSIONS

1. The blood of suprarenalectomized dogs was studied with regard to inorganic phosphorus, blood sugar, pH and alkali reserve.

2. The inorganic phosphorus of the serum rises gradually, increasing with severity of the insufficiency symptoms.

3. The blood sugar shows a decline of great significance with the first signs of weakness. The sugar level may fluctuate after the initial decline but invariably drops in the terminal stages.

4. The $CO_2$ capacity—a measure of the alkali reserve—gradually decreases with the onset of symptoms.

5. The pH persists at normal level until the $CO_2$ capacity falls to from 30 to 33 volumes per cent. At this level the first decline in pH is observed and is seen to accompany the further decrease in $CO_2$ capacity. This condition is indicative of the acidosis which shifts from the compensated state with low $CO_2$ capacity and normal pH, to the uncom-

pensated condition with low $CO_2$ capacity and low pH. The acidosis is in part due to phosphate and sulphate retention.

6. Renal function tests reveal diminished output by the kidney.

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